
**REPORT TO THE SENATE ARMED SERVICES
COMMITTEE AND THE HOUSE OF REPRESENTATIVES
NATIONAL SECURITY COMMITTEE**

on

**Department of Defense
Animal Care and Use Programs 1995**

TABLE OF CONTENTS

	Page
List of Figures	vi
List of Tables	vii
List of Acronyms	viii
Section I Introduction/Overview	I-1
I.1 Requirements for Use of Animals in the DoD	I-1
I.2 DoD Policy Governing Animal Research	I-2
I.3 Scope of Report	I-3
I.3.1 Publicly Accessible Information on Animal Use in the DoD	I-3
I.3.2 Oversight of DoD Animal Care and Use Programs	I-3
I.3.3 Accreditation of DoD Laboratories by AAALAC	I-4
I.3.4 DoD Animal Use and Cost Profiles by Research Category	I-5
I.3.5 DoD Initiatives to Promote Alternative Methods that Replace, Reduce, and Refine the Use of Animals	I-5
Section II Publicly Accessible Information on Animal Use in the DoD	II-1
II.1 Congressional Request Information	II-1
II.2 The FY94 Biomedical Research Database	II-1
II.3 Access and Use of the Biomedical Research Database	II-2
II.4 FY95 Update of the Biomedical Research Database	II-2
Section III Oversight of DoD Animal Care and Use Programs	III-1
III.1 Determination of DoD Needs for Animal Research	III-1
III.2 Oversight of Animal Care and Use Programs and Facilities	III-2
III.2.1 Military Departments	III-2
III.2.2 IACUC	III-2
III.2.3 AAALAC	III-4
III.2.4 Training	III-4
III.2.5 Community Visits	III-5
III.2.6 Office for Protection from Research Risk Oversight	III-5
III.2.7 Additional Oversight	III-5
III.3 Chain of Command over Animal Care and Use Programs	III-6
III.4 Avoidance of Unintended Duplication of Research	III-6
III.5 Avoidance of Unnecessary Research	III-8
III.6 Summary	III-8
Section IV AAALAC Accreditation of DoD Laboratories	IV-1
IV.1 AAALAC Accreditation	IV-1
IV.2 DoD Program Reviews	IV-1
IV.3 DoD AAALAC Accredited Programs	IV-2
IV.4 AAALAC Accreditation Status for U.S. DoD Programs	IV-2
IV.5 AAALAC Accreditation Status for DoD Overseas Programs	IV-2

Section V	DoD Animal Use by Research Category	V-1
V.1	Methods	V-1
V.1.1	Animal Use Profiles	V-1
V.1.2	Animal Use Categories	V-1
V.1.3	USDA Pain Categories	V-2
V.2	Results/Discussion	V-3
V.2.1	General Results	V-3
V.2.2	Animal Use by Service	V-3
V.2.3	Animal Use by Species	V-5
V.2.4	Animal Use by Category	V-9
V.2.5	Animal Use by USDA Pain Category	V-11
Section VI	DoD Initiatives to Promote Alternative Methods that Replace, Reduce and Refine the Use of Animals	VI-1
VI.1	Responsibility	VI-1
VI.1.1	Science and Technology Emphasis on Alternatives to Animal Subjects of Research	VI-1
VI.1.2	Conferences and Workshops on Alternatives to Animal Use	VI-2
VI.1.3	National Research Council, Institute of Laboratory Animal Resources, Educational Programs	VI-2
VI.1.4	Institutional Animal Care and Use Committee Emphasis	VI-3
VI.1.5	Veterinary Staff Expertise and Assistance Visits	VI-3
VI.1.6	Professional Veterinary Training in LAM	VI-3
VI.1.7	AALAS Technician and Laboratory Animal Science Training	VI-4
VI.2	DoD Initiatives to Replace, Reduce and Refine the Use of Animals	VI-4
VI.2.1	Replacement	VI-4
VI.2.1.A	Replacement Using Biochemical or Physical Methods	VI-4
VI.2.1.B	Replacement Using Computer Simulations	VI-5
VI.2.1.C	Replacement Using in vitro Cell Culture	VI-5
VI.2.1.D	Replacement with Non-Mammalian Species and Species Lower on the Phylogenetic Scale	VI-7
VI.2.1.E	Replacement with Human Tissue, or Volunteers as Protocols Progress to Human Trials	VI-8
VI.2.1.F	Replacement with Discarded Tissue from Other Laboratories or Food Processing Plants	VI-8
VI.2.2	Reduction	VI-8
VI.2.2.A	Reduction by Use of Alternative Screening Methods to Study Efficacy in Biological Testing	VI-8
VI.2.2.B	Reduction by Substitution of in vitro or ex vivo Methods	VI-10
VI.2.2.C	Reduction by Substitution of Another Animal Species, or Human Subjects as Protocols Progress into Human Trials	VI-11
VI.2.2.D	Reduction by Substitution of Computer Simulations or Other Technologies	VI-11
VI.2.2.E	Reduction by Sharing Animals between Research Investigations	VI-12
VI.2.3	Refinement	VI-13
VI.2.3.A	Refinement to Protocols that Reduce Pain	VI-13
VI.2.3.B	Refinement to Protocols that Reduce Distress	VI-14
VI.2.3.C	Refinement in Research Models and Animal Alternatives	VI-15
VI.3	Summary	VI-15
Section VII	Glossary	VII-1
Section VIII	References (in order of citation)	VIII-1

Appendix A	DoD Directive on Animal Use	A-1
Appendix B	DoD Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-Sponsored Programs	B-1
Appendix C	DoD Standard IACUC Protocol Format Instructions	C-1
Appendix D	DoD Semiannual Program Review and Facility Inspection Checklist	D-1
Appendix E	DoD Inspector General Recommendations on the Use of Animals in DoD Medical Research Facilities and Contract Research Facilities	E-1
Appendix F	Nonaffiliated IACUC Members Professions	F-1
Appendix G	Dissemination of Information on Animal Care and Use	G-1
Appendix H	IACUC Training and Information	H-1
Appendix I	Journals with DoD Animal Research Publications	I-1
Appendix J	Status of AAALAC Accreditation of DoD Facilities	J-1
Appendix K	Animal Use Categories	K-1
Appendix L	Summary of Animal Use Data by Category	L-1
Appendix M	Walter Reed Army Institute Policy 93-27 - Laboratory Animals Environmental Enrichment Program	M-1
Appendix N	Animal Test Alternatives Publications	N-1
Appendix O	DoD Meetings on Alternative Methods to Animal Use	O-1
Appendix P	Letters from Dr. Martin Stephens	P-1
Appendix Q	National Research Council Fellowship in Alternatives Research at the U.S. Army Edgewood Research, Development, and Engineering Center	Q-1

LIST OF FIGURES

Figure II-1	DoD Biomedical Research Home Page	II-2
Figure II-2	Search Results on Infectious Disease from the BRD	II-3
Figure II-3	Sample of Publicly Accessible Information in the BRD	II-4
Figure III-1	DoD Technology Area Responsibilities	III-7
Figure III-2	Structure of Armed Services Biomedical Research Evaluation and Management Committee	III-7
Figure IV-1	DoD AAALAC Accreditation FY93 - FY95	IV-2
Figure V-1	DoD Animal Use by Year	V-3
Figure V-2	Intramural/Extramural Animal Use by Year	V-3
Figure V-3	Total DoD Intramural and Extramural Animal Use by Service for FY95	V-4
Figure V-4	Total DoD Intramural Animal Use by Service for FY95	V-4
Figure V-5	Total DoD Extramural Animal Use by Service for FY95	V-5
Figure V-6	Total DoD Intramural and Extramural Animal Use by Species for FY95	V-6
Figure V-7	Total DoD Intramural Animal Use by Species for FY95	V-7
Figure V-8	Total DoD Extramural Animal Use by Species for FY95	V-8
Figure V-9	Use of Nonhuman Primates, Dogs, and Cats by Year	V-9
Figure V-10	Total DoD Intramural and Extramural Animal Use by Category for FY95	V-9
Figure V-11	Total DoD Intramural Animal Use by Category for FY95	V-10
Figure V-12	Total DoD Extramural Animal Use by Category for FY95	V-10
Figure V-13	Total DoD Intramural and Extramural Animal Use by USDA Pain Category for FY95	V-11
Figure V-14	Total DoD Intramural Animal Use by USDA Pain Category for FY95	V-12
Figure V-15	Total DoD Extramural Animal Use by USDA Pain Category for FY95	V-12

LIST OF TABLES

Table I-1	Summary of DoD Animal Use Statistics	I-5
Table I-2	Examples of DoD Initiatives for Replacement, Reduction, and Refinement of the Animals Used in Research	I-6
Table IV-1	DoD FY95 AAALAC Accreditation Status	IV-2
Table V-1	Animal Use Categories	V-2
Table V-2	USDA Pain Categories (USDA APHIS form 7023)	V-2

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
CRISP	Computer Retrieval Information of Scientific Projects
DDR&E	Director, Defense Research and Engineering
DoD	Department of Defense
DTIC	Defense Technical Information Center
ELISA	Enzyme Linked Immunosorbent Assay
FEDRIP	Federal Research in Progress
FY	Fiscal Year
IACUC	Institutional Animal Care and Use Committee
IG	Inspector General
ILAR	Institute of Laboratory Animal Resources
JDL	Joint Directors of Laboratories
JTCG	Joint Technology Coordinating Groups
LAM	Laboratory Animal Medicine
MATRIS	Manpower and Training Research Information Services
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OPRR	Office for the Protection from Research Risks
OSD	Office of the Secretary of Defense
PADPRP	Poly (ADP-ribose) Polymerase
PCR	Polymerase Chain Reaction
PHS	Public Health Service
RDT&E	Research, Development, Test, and Evaluation
S&T	Science and Technology
SEB	Staphylococcus Enterotoxin B
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
WRAIR	Walter Reed Army Institute of Research

SECTION I

INTRODUCTION/OVERVIEW

This is the Fiscal Year (FY) 1995 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 (IACUC), 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 FY95. 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, & 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 over 120 thousand individuals in endemic areas of Argentina against the ravages of Argentinean Hemorrhagic Fever; DoD-developed Venezuelan Equine Encephalitis (VEE), Eastern Equine Encephalitis (EEE), and Western Equine Encephalitis (WEE) 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 biowarfare defense, where animal-based studies are particularly critical. Ethical concerns, as well as regulatory requirements of the Food and Drug Administration, 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. We have 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 developed and implemented a new 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 1976 (P.L. 94-279) and 1985 (P.L. 99-198), as well as the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals*, (fifth edition, 1985, NIH 86-23) 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 genus *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 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. Service and DoD animal use by research categories (Section V), and
- e. DoD initiatives to promote alternative methods that replace, reduce, or refine animal use (Section VI).

I.3.1 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 NIH Computer Retrieval Information of Scientific Projects System. The DoD Biomedical Research Database 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 FY95 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.3.2 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 revised DoD Directive 3216.1 (1995) continues to specify that DoD IACUCs 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 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 E). 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 IACUC members conducting the inspection, 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.

The revised DoD Directive 3216.1 (1995) clarifies composition, membership, and training requirements of the IACUC. The 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 their 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). Her 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.3.3 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 37 DoD animal facilities worldwide, of these 33 (89%) are accredited, a record that far exceeds the approximately 46% accreditation rate for civilian research laboratories registered with the USDA. All other DoD facilities have applied for accreditation.

AAALAC's philosophy of accreditation is steadily evolving from an emphasis on physical facilities (engineering standards) to a more comprehensive evaluation of the total laboratory animal care and use program (performance standards). Consequently, research units that were previously regarded as unaccreditable until major facility renovations or upgrades were completed have now been accredited by AAALAC. The Inspector General's "Review of the Use of Animals in the Department of Defense Medical Research Facilities" confirmed the effectiveness of animal husbandry programs in DoD facilities and concluded that although not all facilities were AAALAC accredited, animals in DoD facilities were maintained in healthy environments and treated humanely. As stated in the report, "The inspection teams were completely satisfied with the health and welfare of the animals in DoD research facilities.... All the personnel assigned the care of the animals were competent, interested, and committed to the humane care of the animals."

I.3.4 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 1995, the DoD used 431,879 animals which is a 28% decrease from FY94. Of these, 28,245 (7%) were USDA reportable species as defined in the Animal Welfare Act of 1985. Table I-1 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.

Table I-1 Summary of DoD Animal Use Statistics

Total Animal Use by Species	% of Total
Rodents, fish, amphibians and birds	96.51
Rabbits	0.92
Farm animals (i.e., sheep, pigs, cows, horses)	1.55
Dogs, cats, nonhuman primates, marine mammals	0.66
Other	0.36

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

Total Animal Use by Category	% of Use
Medical RDT&E	84.9
Non-Medical RDT&E	4.2
Clinical Investigation	4.9
Adjuncts/Alternatives	3.9
Training & Instructional	1.8
Breeding Stock	< 1
Classified Secret or Above	< 1
Offensive Weapons Development	0

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

I.3.5 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 uses of animals. Approximately 170 examples of DoD efforts to replace, reduce, and refine the use of animals in research are reviewed in Section VI. Animal research is an essential part of the scientific process, but it is only initiated after due consideration of alternatives. The DoD uses a 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, 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. The U.S. Army Medical Research and Materiel Command has established a major objective to develop replacement, reduction, and refinement strategies for the use of animals in research. In addition, the Department sponsors conferences and workshops to promote alternatives to animal research. The DoD sponsors a 5-year grant with the Institute of Laboratory Animal Resources of the National Research Council to develop institutional training materials, education, and publications in support of DoD laboratory animal care and use programs. The Institutional Animal Care and Use Committee process also includes a strong emphasis on consideration of alternatives in all new protocols. Table I-2 describes several examples of new Department initiatives that replace, reduce and refine the use of animals.

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

Table I-2 Examples of DoD Initiatives for Replacement, Reduction, and Refinement of the Animals Used in Research

Replace nonhuman primates with rats for Hepatitis E Virus (HEV) bioassay.
Use of guinea pigs will preclude the requirement for nonhuman primates in all but the most critical pathogenesis and protective efficacy studies.
Cell cultures used to replace mice and rats to test inhibitors of cAMP degradation.
Reduction in numbers of swine and goats by conducting power analysis to determine minimal numbers of animals to use in surgical studies.
Reduction in numbers of mice through use of computer modeling of potential peptide antigens to determine if conformation sequence is analogous to native protein.
Use of a slow release subcutaneously placed estrogen capsule avoids the need for daily intramuscular injections in rats.
Development of fish (rainbow trout, zebra danio fish and medaka) as predictive models for epigenetic carcinogens has reduced mammalian animal use in carcinogenesis studies.

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.

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 [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 of 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 Internet on October 1, 1995. It is located on the Manpower and Training Research Information Services (MATRIS) home page.

II.2 THE FY94 BIOMEDICAL RESEARCH DATABASE

The data in the FY94 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 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 FY94 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 FY94 BRD contained 790 summaries and was made accessible to the public on October 1, 1995. 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:

Accession Number: Identification number given by the database.

POC/Author: Primary contact for the work unit is usually the Public Affairs Office.

POC Address: The complete mailing address of the POC.

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.

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 (i.e., 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 through the MATRIS home page at:

<http://dticam.dtic.mil/www/welcome.html>

or directly at:

<http://dticam.dtic.mil/www/dodbr/dodbrfrm.html>

The BRD home page shown in Figure II-1 is a searchable database. To perform a search, enter a specific search topic in the search window and click on Do Search or press Enter. The results of the search will produce a hypertext list of titles (Figure II-2). To access a particular summary, click on the specific title and a summary will appear (Figure II-3). In addition, a list of all the summaries can be accessed by selecting [View all titles](#).

II.4 FY95 UPDATE OF THE BIOMEDICAL RESEARCH DATABASE

The DoD will make all FY95 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

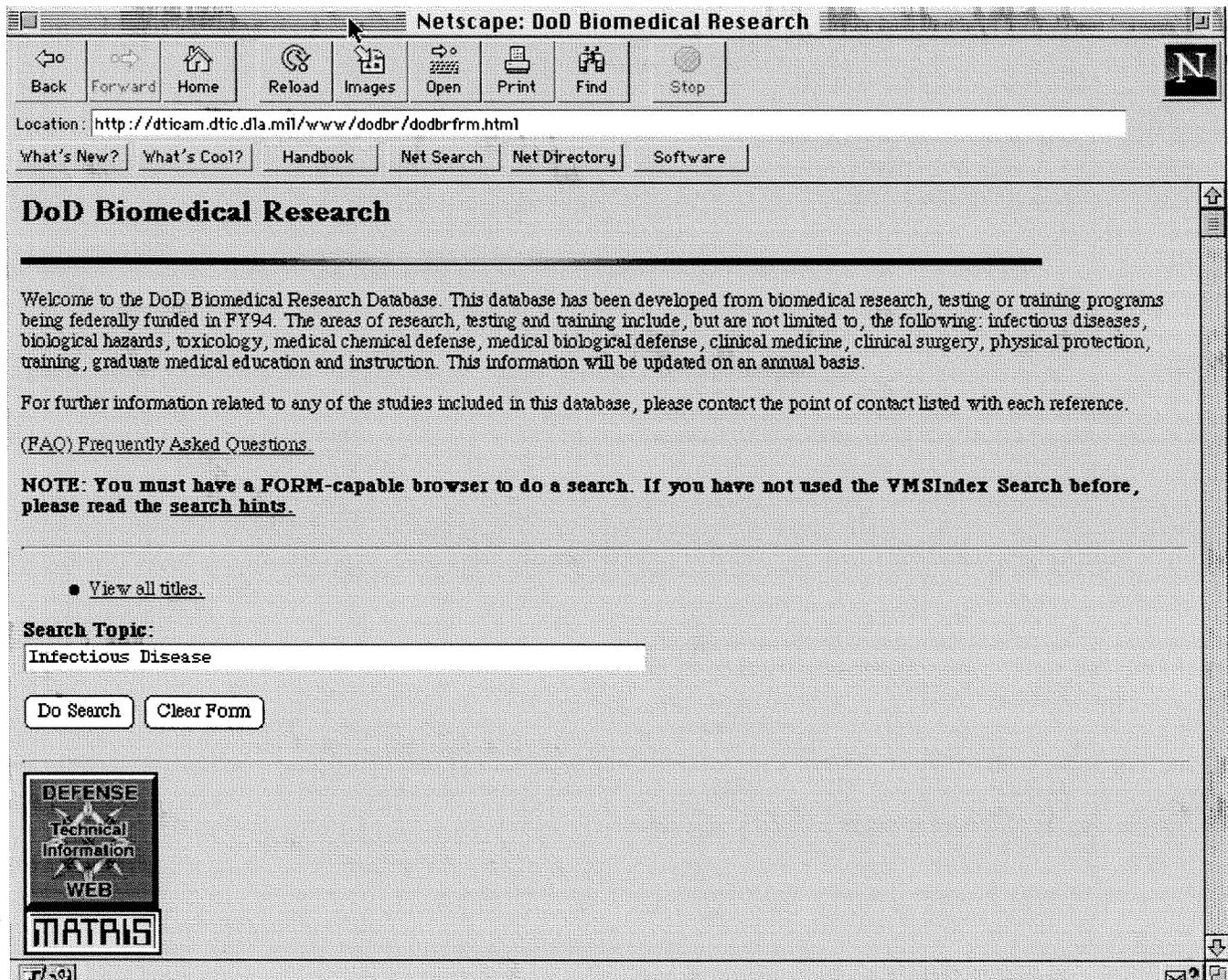


Figure II-1 DoD Biomedical Research Home Page

training work for the FY95 BRD. The cost of FY95 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, 1996.

Netscape: Search all dodbr html files on MATRIS

Location: <http://dticam.dtic.dla.mil/htbin/dodbrquery>

What's New? What's Cool? Handbook Net Search Net Directory Software

infectious disease

There are 24 items found:

1. [Identification and Control of Insect Vectors of Infectious Diseases](#)
2. [Animal-Facilitated Clinical Medicine Studies in Support of Graduate Medical Education](#)
3. [Prophylactic and Therapeutic Measures Against Infectious Diseases of Military Importance Endemic to Southeast Asia](#)
4. [Evaluation of the Potential Threat of Arboviral and Other Infectious Diseases to Deployed Military Troops in the Amazon Basin Region of Peru](#)
5. [Purification and Characterization of the Soluble Toxin and Immunosuppressive Factor\(s\) of Mycobacterium Ulcerans](#)
6. [Pathogenesis of a Newly Isolated Viruslike Infectious Agent in Experimental Animals](#)
7. [Risk Assessment and Establishment of the Murine Model for Brucellosis](#)
8. [Role of Macrophages in VEE Pathogenesis - A Molecular Approach](#)
9. [Regulation of B Cell Maturation](#)
10. [Burn Injuries](#)
11. [Prepare Field Sites for Evaluating Malaria Vaccines](#)
12. [Improve Isolation and Identification of Malaria](#)
13. [Characterize the Biochemical, Physical, Antigenic, and Molecular Structure of Leishmanial Macromolecules](#)
14. [Investigate the Genetics and Physiology of Yersinia Pestis](#)
15. [Design, Characterize, and Evaluate Medical Countermeasures \(i.e., Vaccines, Immune Serum\)](#)
16. [Evaluate Genetically Engineered VEE Vaccines](#)
17. [Evaluate Insect Populations and Subpopulation Diseases](#)
18. [Vaccines Vectored - Korean HF Vaccine](#)
19. [Characterize, Design, and Evaluate Methodology for Improved Diagnosis and Cultivation of Viruses of Military Importance](#)
20. [Dengue Recombinant Subunit Vaccine Development](#)
21. [Rift Valley Fever and Other Arboviral Infections](#)
22. [Vaccine Induced Enhancement of Equine Infectious Anemivirus \(EIAV\) Application and Disease](#)
23. [Production of Cytokine-Specific Monoclonal Antibodies that Modulate Immune and Inflammatory Processes](#)
24. [Vaccine-induced Enhancement of EIAV Replication and Disease](#)

Figure II-2 Search Results on Infectious Disease from the BRD

Title: Treatment and Prevention of Sepsis and Septic Shock

FY94 Funding: \$444,500

Primary Contact: Public Affairs Office

Organization: Naval Medical Research Institute

Address: 8901 Wisconsin Avenue

City: Bethesda

State: MD

Zip: 20889-5607

Performing Organization: Naval Medical Research Institute

Address: 8901 Wisconsin Avenue

City: Bethesda

State: MD

Zip: 20889-5607

Objective and Approach:

Objective: The objective of this work is to evaluate candidate therapies to lessen the morbidity and mortality in septic shock in combat casualties. Sepsis and septic shock are the result of a profound host response to bacterial infection. In sepsis, high concentrations of powerful mediators induce the adhesion and activation of inflammatory cells and consequent widespread tissue damage. To date therapies directed at ablating this inflammatory sequence have been shown to be promising in animal models but have proven ineffective in clinical trials. This proposal is directed at evaluating treatments to specifically modulate the inflammatory response and prevent the unwanted, promiscuous adhesion and activation of inflammatory cells. The goal is to prevent inflammation-induced damage to tissues in areas remote to the infection yet retain the bactericidal benefits at the site of infection. Specifically, the aim of this proposal is to evaluate the efficacy of antisense oligonucleotide agents directed at preventing the expression of specific molecules on the surface of cells thus preventing the adhesion of inflammatory leukocytes. A second aim is to evaluate the efficacy of carbohydrate-based agents to inhibit the binding of adhesion molecules to their carbohydrate receptors, thereby preventing the adhesion of inflammatory leukocytes. Both of these therapies offer the potential of being directed at highly specific targets and only in specific tissues. In addition, the regulation of lipopolysaccharide-induced genes during sepsis will be studied to develop new targets to prevent sepsis.

Approach: Antisense oligonucleotides complementary to target sequences in the mRNA and pre-mRNA or various cell adhesion molecules will be tested in cell culture assays in another work unit. The most active oligonucleotides will be evaluated for their ability to prevent neutrophil adhesion in the lungs of lipopolysaccharide stimulated mice. In addition, the oligonucleotides will be tested for their ability to enhance survival in a galactosamine-sensitized low-dose lipopolysaccharide lethality model and for their ability to prevent sepsis in animals. Various delivery systems will also be evaluated for the potential to enhance the specificity and to improve morbidity and mortality. The adhesion of leukocytes to sites in the vascular bed depends on the specific binding of pairs of cell surface bound adhesion molecules. In this binding, the adhesion molecules recognize and bind to specific carbohydrates on the complementary cell. In this proposal, various carbohydrate-based inhibitors will be used to disrupt the adhesion of leukocytes. They will be evaluated in the same animal models for the ability to prevent leukocyte adhesion and lessen morbidity and mortality in sepsis. Heparin analogues proven to be effective in improving microvascular patency in hemorrhagic shock will be evaluated in these models as well.

Indexing Terms:

antisense
oligonucleotides
inflammation
cell adhesion
sepsis
septic shock
carbohydrate
heparin
leukocytes
gene expression
animals
mice
LPS

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to the use of animals in research and was reviewed and approved by the Institute's Animal Care and Use Committee.

Figure II-3 Sample of Publicly Accessible Information in the BRD

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, and 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 a way that is in full compliance with applicable laws, regulations, and guidelines.

III.1 DETERMINATION OF DoD NEEDS FOR ANIMAL RESEARCH

Determining research needs and research plans is a comprehensive process integrated into DoD's planning, programming and budgeting processes. 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 the Congress with significant detail of every research project's past accomplishments, planned accomplishments and future plans.

Each DoD research laboratory tailors its organization, staffing, and related infrastructure within available resources to best meet its science

and technology mission and to support each Commander's accountability, responsibility and authority. In October 1995, the Department implemented the use of 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 complete and thorough Institutional Animal Care and Use Committee (IACUC) review for all animal use proposals. Although the specific procedural elements and processes of individual protocol review may differ in minor ways from facility to facility, the general submission, review and approval processes are summarized as follows.

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 non-duplication of previous or ongoing research. Previously, this search was performed only on the Defense Technical Information Center (DTIC) database. DTIC maintains a database of ongoing and completed DoD research at the work unit level of detail. The Standard Protocol Format requires that "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." 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 the local 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 deal with alternatives to painful procedures. Additional pertinent know-

ledge 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 require the utilization of Defense resources, individual protocols are reviewed for factors such as military relevancy, necessity, scientific merit, and relative research priority. Such reviews are normally conducted within the laboratory's command-and-control structure and are routinely 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 (a) the study is based on sound scientific principles; (b) the number of animals used is the minimum required to achieve the purpose; (c) the phylogenetically lowest species of animal is selected as the appropriate model; (d) there is appropriate use of analgesics and anesthetics, or if required, there is adequate scientific justification if not used; (e) the research is not unnecessarily duplicative; (f) the personnel conducting the research are qualified by training and experience to conduct the research; and (g) the scientific question to be answered 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 International (AAALAC).

III.2.1 Military Departments

Each Military Department has a component or 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 programs in the Navy resides in the Office of the Surgeon General of the Navy. Oversight is accomplished by a specialty trained LAM veterinarian assigned to the Naval Medical Research and Development Command, who also serves the Health Services Education and Training Command (Clinical Investigations), and the Inspector General at the Naval Bureau of Medicine and Surgery. Air Force oversight responsibility rests with a specialty trained LAM veterinarian assigned to the HQ, Air Force Medical Operations Agency, Clinical Investigations & Life Sciences Division, Office of the Air Force Surgeon General, and with the Office of the Director of Medical Inspection, Air Force Inspection Agency.

III.2.2 IACUC

The backbone of the review procedures for all DoD animal-based research is the IACUC review of the research proposal or protocol. DoD Directive 3216.1 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

currently chaired by an individual with credentials and experience appropriate to the post, typically a senior physician, scientist, or veterinarian. The DoD Directive 3216.1 (1995) (Appendix A) clarifies the composition, membership, and training requirements of the IACUC. The revised Directive (1995) 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 diverse backgrounds/professions of the nonaffiliated and alternate nonaffiliated IACUC members are provided in Appendix F. Currently, 28% of the nonaffiliated members are private sector civilians, 49% are civilians employed by the federal government, and 23% are military. In accordance with the Directive, these members represent the community and are not affiliated with the research facility. Full compliance with the new 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 on October 1, 1995.

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 Corp's 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.

It is a proactive Department policy that nonaffiliated members are encouraged to perform unannounced site visits of animal care facilities in addition to full participation in all discussions and votes on all research proposals. At least 20 unannounced visits to Department animal facilities by nonaffiliated members of DoD IACUCs were reported in FY95.

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. Each DoD IACUC is currently using the new standardized checklist during their semiannual program reviews. The IACUC prepares written reports of its evaluations and submits them to the Institutional Official, usually the facility commander. Reports specifically address compliance with the AWA, and identify any departures from the Act to include an explanation for the departure. The report must distinguish between significant 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 concerning humane care and treatment of animals arise. DoD facilities have developed a wide variety of proactive and innovative mechanisms to both

inform the public on how to contact responsible individuals as well as programs to ensure that those who work with animals are fully apprised of the requirement to provide humane and ethical care (Appendix G). Additionally, IACUCs make recommendations to the Institutional Official regarding any aspect of the research facility's animal program, facility, or personnel training; 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 the 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 DoD's participation are provided in Section IV.

III.2.4 Training

The DoD provides extensive veterinary and animal care services for DoD 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 current interpretations and implementing regulations of the AWA. The DoD sponsors formal post-doctoral training programs for veterinarians in LAM, including a nationally recognized in-house 4-year residency program culminating in specialty board eligibility for

certification in the American College of Laboratory Animal Medicine. Some DoD veterinarians attend various university post-graduate LAM training programs resulting in a masters degree 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 post-graduate Masters of Public Health in Laboratory Animal Medicine at the Uniformed Services University of the Health Sciences. This outstanding new program will provide the Department with a new source of laboratory animal medicine 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 28 years, the DoD has trained over 3,250 animal care specialists. 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 each is fully cognizant of the numerous responsibilities of IACUC members under the provisions of the AWA. The 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 on 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 H). 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 H.

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, either at the facility, post, or base. Visits by the public or the press are arranged and coordinated through the appropriate public affairs office. DoD facilities are visited by various special interest groups including community and civic groups; animal welfare or animal advocates, groups or individuals; dignitaries, academics and teachers; local, state, and national politicians; congressional members and staff; elementary to post-doctoral students; print and electronic journalists and authors, etc. Consequently, a greatly diversified range of individuals are 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 NIH'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 is 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, but are not limited to, 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, IG complaints can be made anonymously, with no requirement to identify oneself in the registering of a complaint. 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 the revised 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 conducting 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 between 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 the 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, 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 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 (JTCCGs), 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

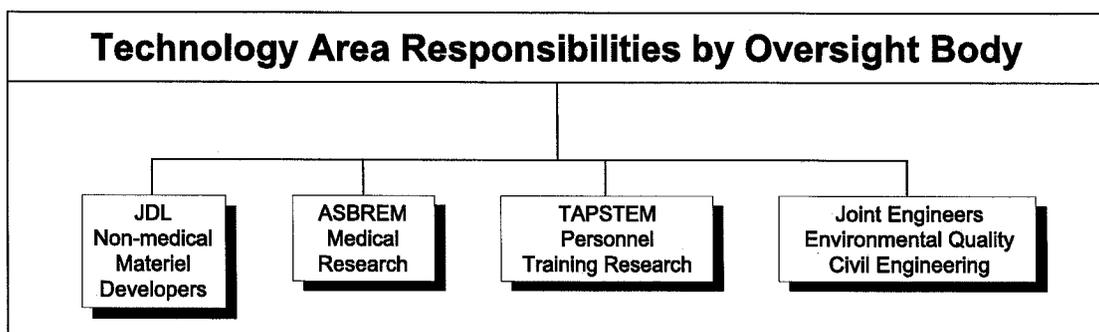


Figure III-1 DoD Technology Area Responsibilities

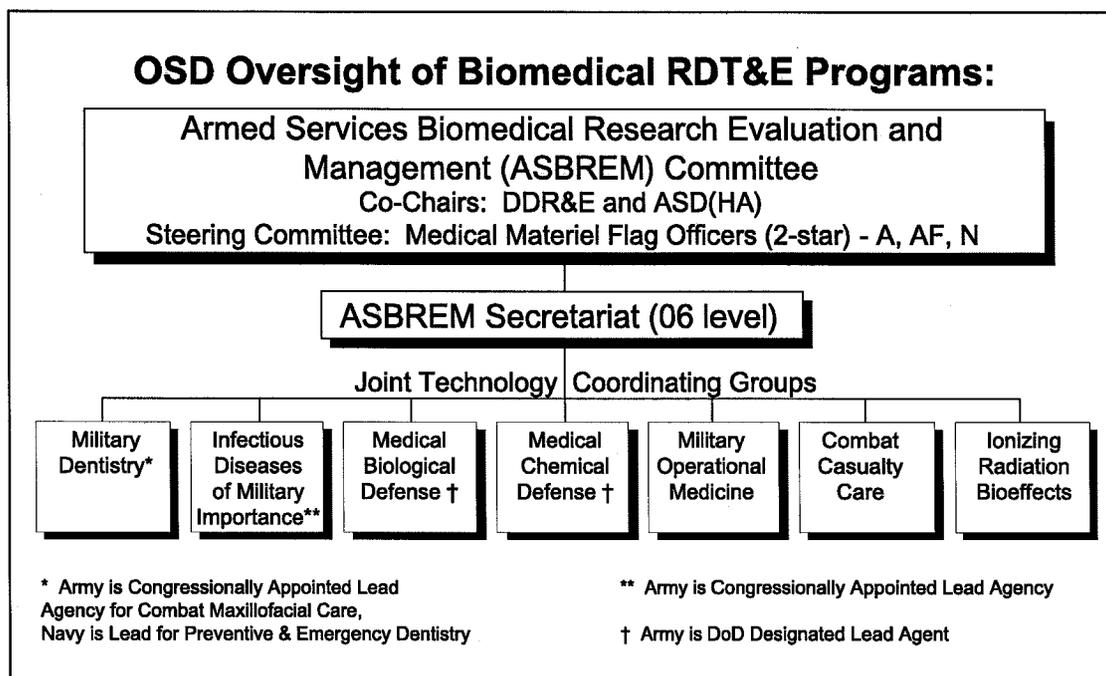


Figure III-2 Structure of Armed Services Biomedical Research Evaluation and Management Committee

technology areas. For example, the ASBREM Committee is supported by the JTCGs (Figure III-2) and the JDL is supported by separate technology panels.

In addition to these formal coordination and review processes to eliminate unintended duplication of research, 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 in proportion to 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 impacts 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 or presentation to a professional meeting (Appendix I). Peer-reviewed journals critique the research during the review process leading to an overall enhancement of the research process as well as validating 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 also 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, as well as from 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 is 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. Over the past decade, the DoD in concert with the 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. Individual IACUCs provide oversight of animal care and use programs and research. Additionally, IACUCs provide training and information about animal care and use, and ensure the humane use of animals in research. Each DoD facility's IG is 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. 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 the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accreditation. 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 Institutes of Health (NIH) 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. 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 for the evaluation of many of its programs, such as drug screening laboratories, and review of military medical facilities by the Joint Commission for Accreditation of Health Organizations. 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 the remote locale, limited scope of operations, or host nation sovereignty. In these cases, equivalency standards can apply and be effectively monitored. The Joint Service Regulation and Service-conducted inspections of facilities implement the requirements of the Animal Welfare Act and the NIH *Guide for the Care and Use of Laboratory Animals*.

The DoD is committed to continuing its full participation in the AAALAC accreditation process as the external peer review evaluation method 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 3 years (Figure IV-1). There are 37 DoD animal facilities worldwide that use animals; of these, 33 (89%) are AAALAC accredited. This increase reflects DoD's commitment to accrediting all of its animal care and use programs.

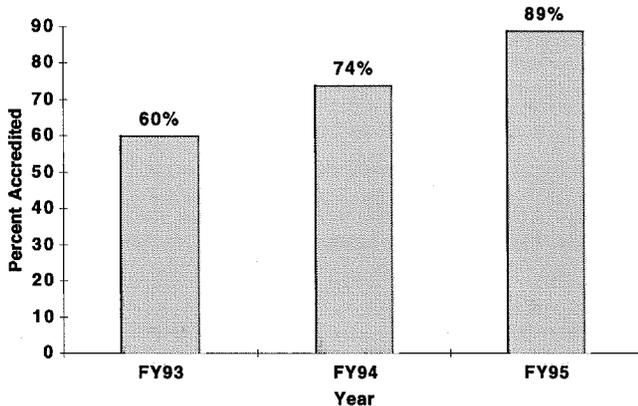


Figure IV-1 DoD AAALAC Accreditation FY93 - FY95

IV.4 AAALAC ACCREDITATION STATUS FOR U.S. DoD PROGRAMS

There are 33 programs in the U.S. that maintain animals for research, testing, or training for the DoD. Table IV-1 shows that of the 33 DoD programs in the U.S., 91% are accredited by AAALAC. This compares very favorably with the accreditation rate for the 1,324 United States Department of Agriculture registered and active animal facilities; 604, or 46%, are accredited by AAALAC. The three remaining DoD programs in the U.S. that are not yet accredited have applied for accreditation. 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 J provides additional information on AAALAC accreditation by program.

The AAALAC philosophy of accreditation is steadily evolving from an emphasis on physical

Table IV-1 DoD FY95 AAALAC Accreditation Status

AAALAC Status	U.S. DoD Programs	Overseas DoD Programs
Accredited	30	3
Application Submitted	3	1
Total	33	4

facilities (engineering standards) to a more comprehensive evaluation of the total laboratory animal care and use program (performance standards). Facilities are still an important consideration in the accreditation process, but are no longer the paramount element. Consequently, research units that were previously regarded as unaccreditable until major facility renovations or upgrades were completed are now actively pursuing AAALAC accreditation on the basis of comprehensive, high quality laboratory animal care and use programs. The lack of accreditation does not imply that animals are exposed to unhealthy conditions.

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 (75%) of its four overseas laboratories. The Naval Medical Research Detachment in Lima, Peru, is the first laboratory in South America, to receive AAALAC accreditation. The Naval Medical Research Unit #2 in Jakarta, Indonesia, is the first DoD laboratory in Southeast Asia to be accredited, and the Naval Medical Research Unit #3 in Cairo, Egypt, is the first laboratory in Africa to be accredited.

SECTION V

DoD ANIMAL USE BY RESEARCH CATEGORY

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) 1995.

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 the Animal and Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (i.e., 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 FY95 were 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 is injected, manipulated, handled, or administered medication and/or experimental compounds within a given work unit, protocol, or program. Animals on hand during FY95, 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 K. 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

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 anesthetics, 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. In addition, the primary investigator must write a justification for all procedures for animals in Columns D and E.

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

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.

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 431,879 animals used in FY95 which is a 28% decrease from FY94 and a 22% 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 warmblooded animal, as the Secretary may determine..." Therefore, only 7% (28,245) of the animals used by the DoD in FY95 are considered USDA reportable species.

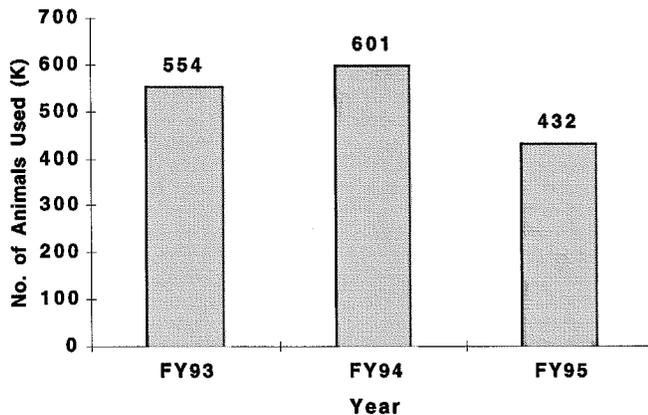


Figure V-1 DoD Animal Use by Year

In FY95 228,525 animals were used in intramural research programs and 203,354 were used in extramural grants or contracts (Figure V-2). There was a 15% and 39% decrease in FY95 intramural and extramural animal use, respectively. The decreased use of animals by extramural programs accounts for 77% of the total FY95 decrease. By their very nature, extramural research programs have the greatest fluctuation in the

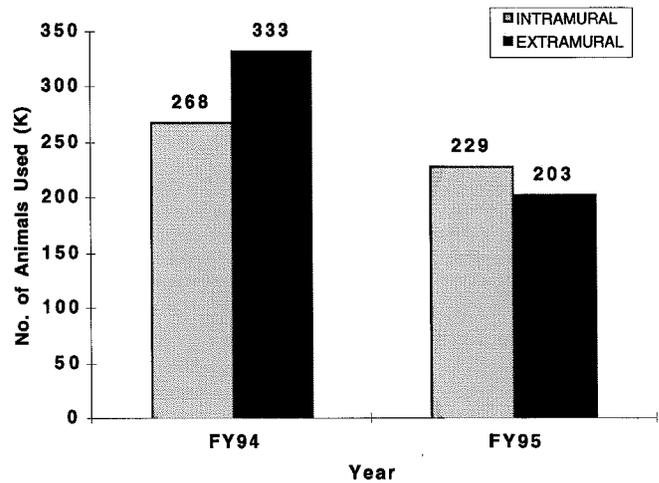


Figure V-2 Intramural/Extramural Animal Use by Year

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 (i.e., 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 the Breast Cancer Research Program in which 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 or decrease in the number of research projects.

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 FY95, the Army used 74% of the DoD total animal use, 58% of the total intramural animals and 92% of total extramural animals.

TOTAL = 431,879

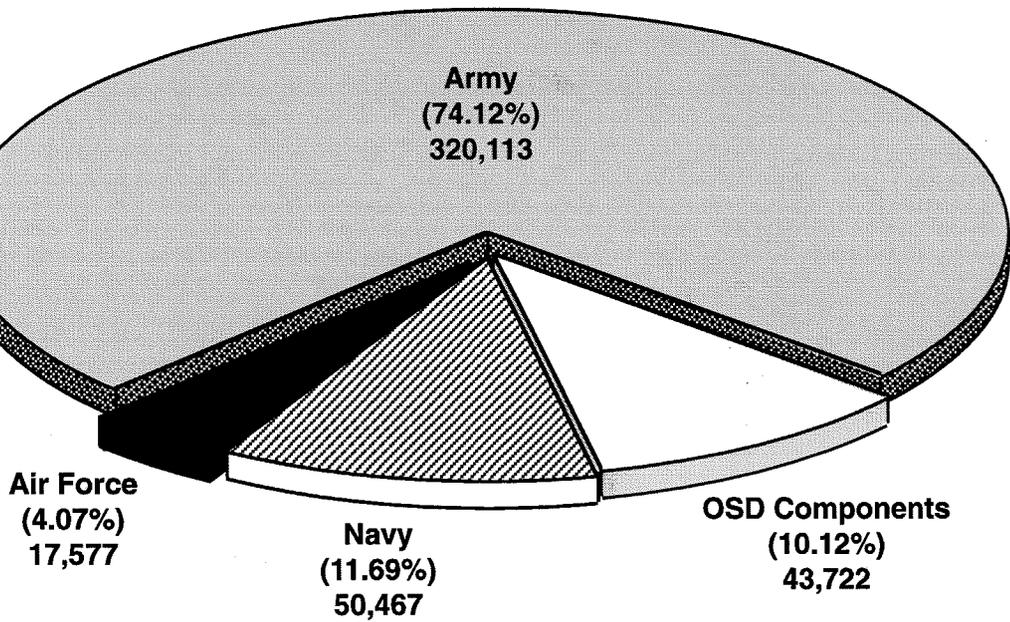


Figure V-3 Total DoD Intramural and Extramural Animal Use by Service for FY95

TOTAL = 228,525

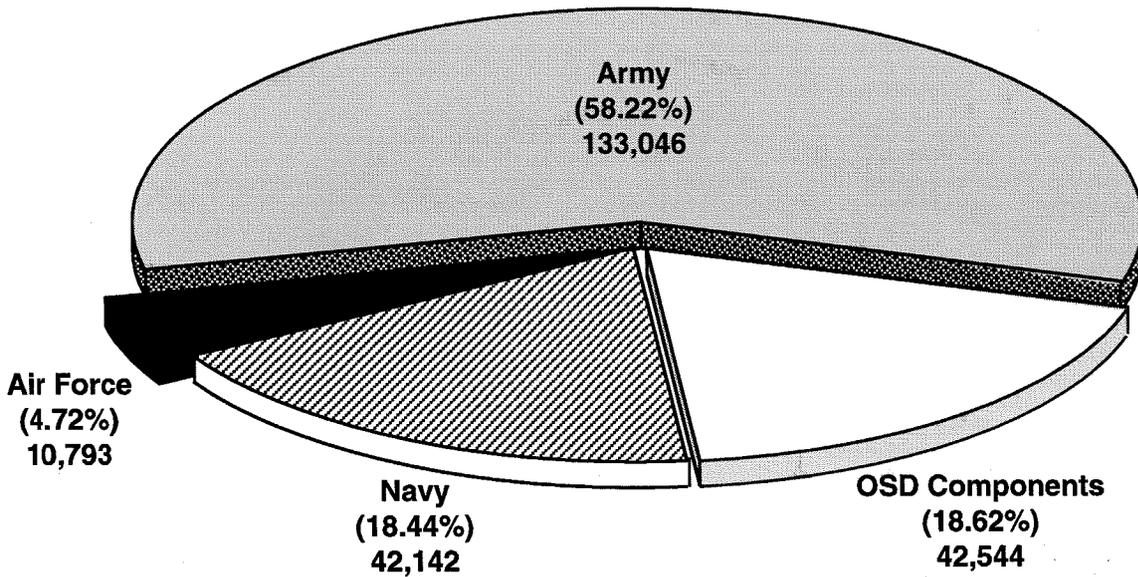


Figure V-4 Total DoD Intramural Animal Use by Service for FY95

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

TOTAL = 203,354

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

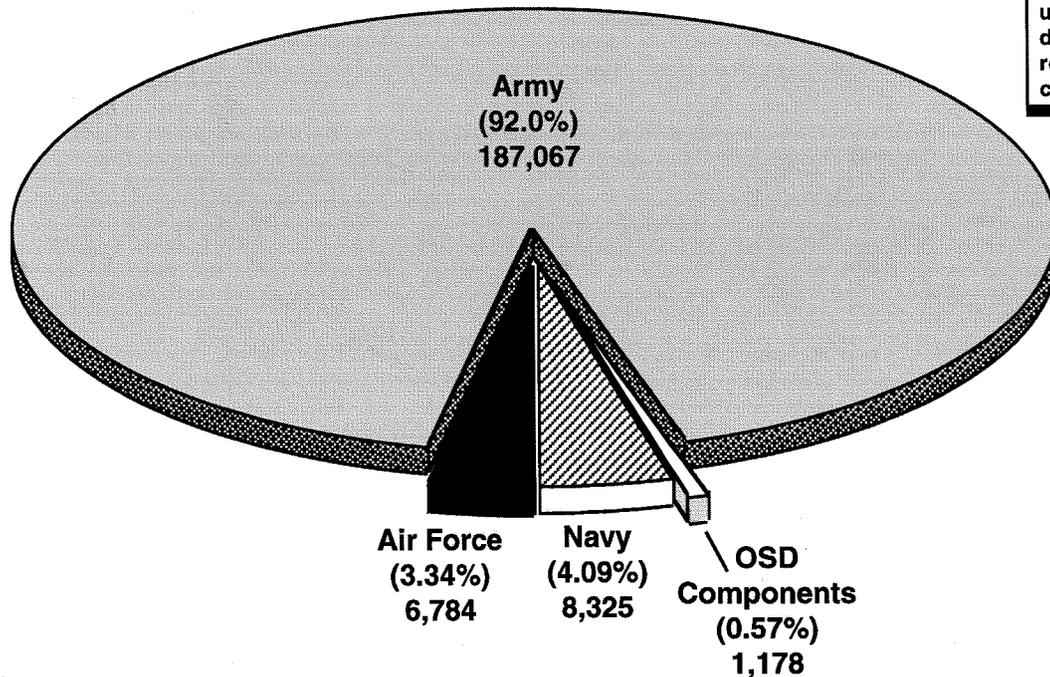


Figure V-5 Total DoD Extramural Animal Use by Service for FY95

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 medical biological defense and nutrition studies. The Command is responsible for greater than 85% of the DoD Medical Research, Development, Test and Evaluation programs. In addition, the Army has an ongoing responsibility to manage the congressionally mandated Breast Cancer Research Program. The Army had a 30% decrease in the number of animals used in FY95 resulting from a 105,196 decrease (36%) in the number of animals used in the Army's extramural research programs.

The Navy used 12% of the DoD total animal use, 18% of the total intramural animals and 4% of total extramural animals. In FY95, the Navy used 5,666 more animals, with the majority of these animals used in intramural research programs. There was a slight decrease in the Navy's extramural animal use for FY95.

The Air Force used 4% of the DoD total animal use, 5% of the total intramural animals and 3% of total extramural animals. The Air Force

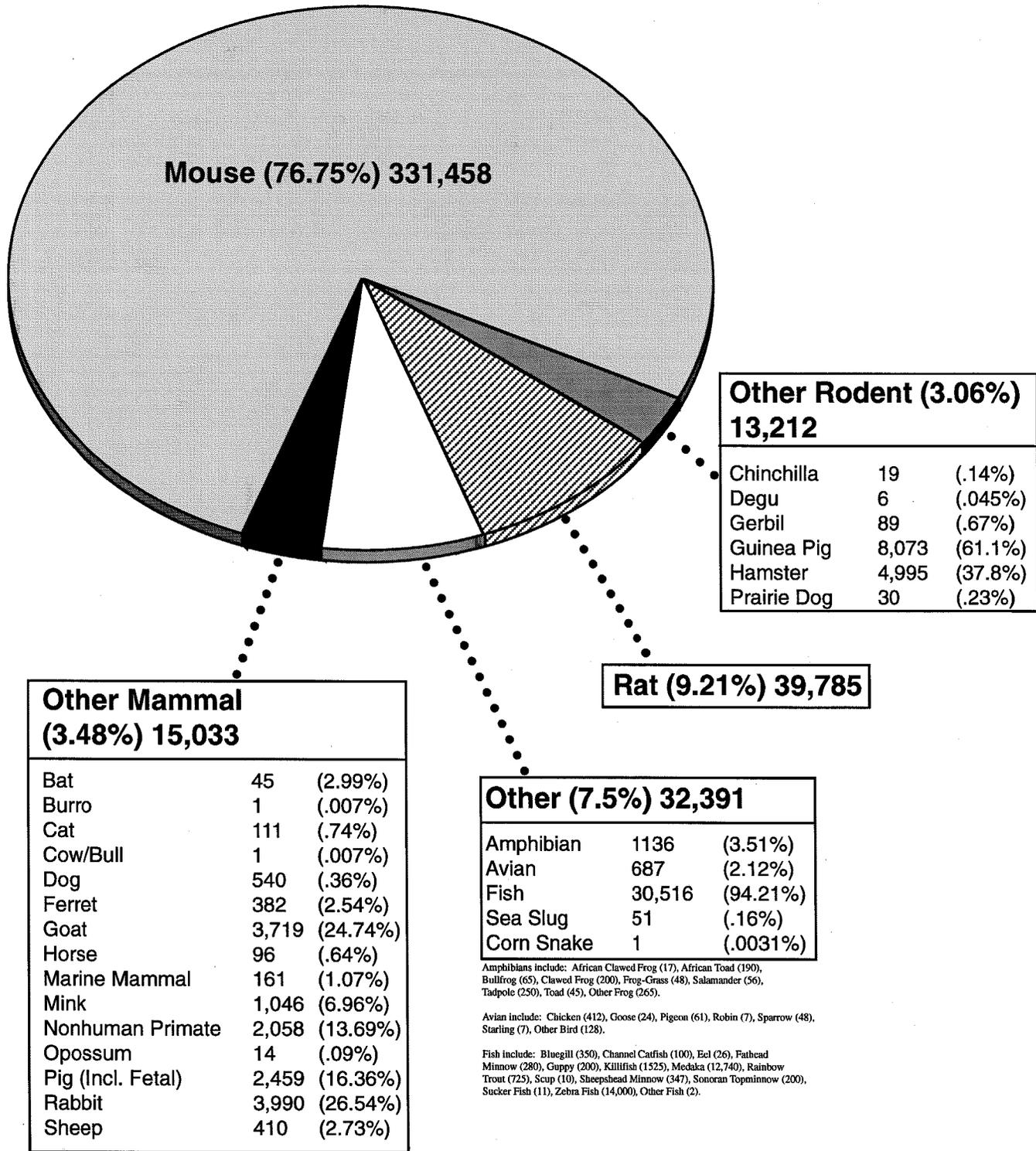
decreased the number of animals used in research by 20,401 animals (54%) in FY95. Most of this decrease (18,852) was in the Air Force's extramural research projects.

The Office of the Secretary of Defense (OSD) components are the Uniformed Services University of the Health Sciences, Advanced Research Projects Agency, Armed Forces Radiobiology Research Institute, and Armed Forces Institute of Pathology. OSD components used 10% of the DoD total animal use, 18% of the total intramural animals and less than 1% of total extramural animals. There was a 26% (15,259) decrease in the use of animals for the OSD components in FY95.

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 FY95. The majority (~97%) of animals used by the DoD, both intramurally and extramurally, were rodents, birds, amphibians and fish. The numbers of both nonhuman primates and dogs and cats decreased in FY95 (Figure V-9).

TOTAL = 431,879



Marine Mammals include: Beluge Whale (9), Bottlenose Dolphin (87), California Sea Lion (14), Commerson's Dolphin (2), Common Dolphin (1), False Killer Whale (9), Fin Whale (1), Harbor Seal (4), Killer Whale (5), North Elephant Seal (7), Pilot Whale (1), Risso's Dolphin (8), Sperm Whale (2), Steller Sea Lion (4), Weddel Seal (6), White Side Dolphin (1).

Nonhuman Primates include: African Green Monkey (14), Baboon (28), Bonnet Monkey (2), Cynomolgous Monkey (190), Patas Monkey (16), Pigtail Monkey (103), Rhesus Monkey (1527), Squirrel Monkey (72), Other Monkey (106).

Amphibians include: African Clawed Frog (17), African Toad (190), Bullfrog (65), Clawed Frog (200), Frog-Grass (48), Salamander (56), Tadpole (250), Toad (45), Other Frog (265).

Avian include: Chicken (412), Goose (24), Pigeon (61), Robin (7), Sparrow (48), Starling (7), Other Bird (128).

Fish include: Bluegill (350), Channel Catfish (100), Eel (26), Fathead Minnow (280), Guppy (200), Killifish (1525), Medaka (12,740), Rainbow Trout (725), Scup (10), Sheepshead Minnow (347), Sonoran Topminnow (200), Sucker Fish (11), Zebra Fish (14,000), Other Fish (2).

Figure V-6 Total DoD Intramural and Extramural Animal Use by Species for FY95

TOTAL = 228,525

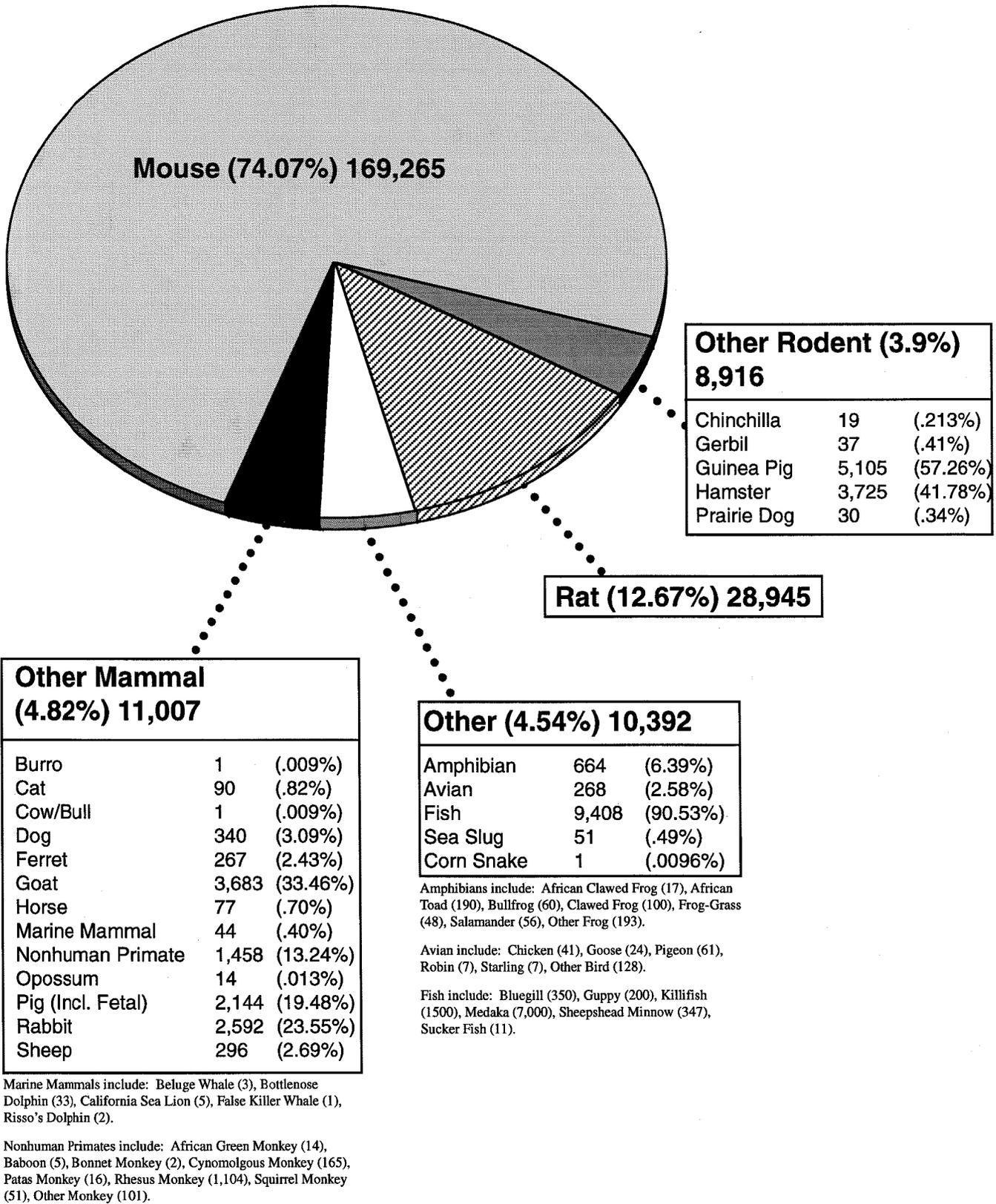


Figure V-7 Total DoD Intramural Animal Use by Species for FY95

TOTAL = 203,354

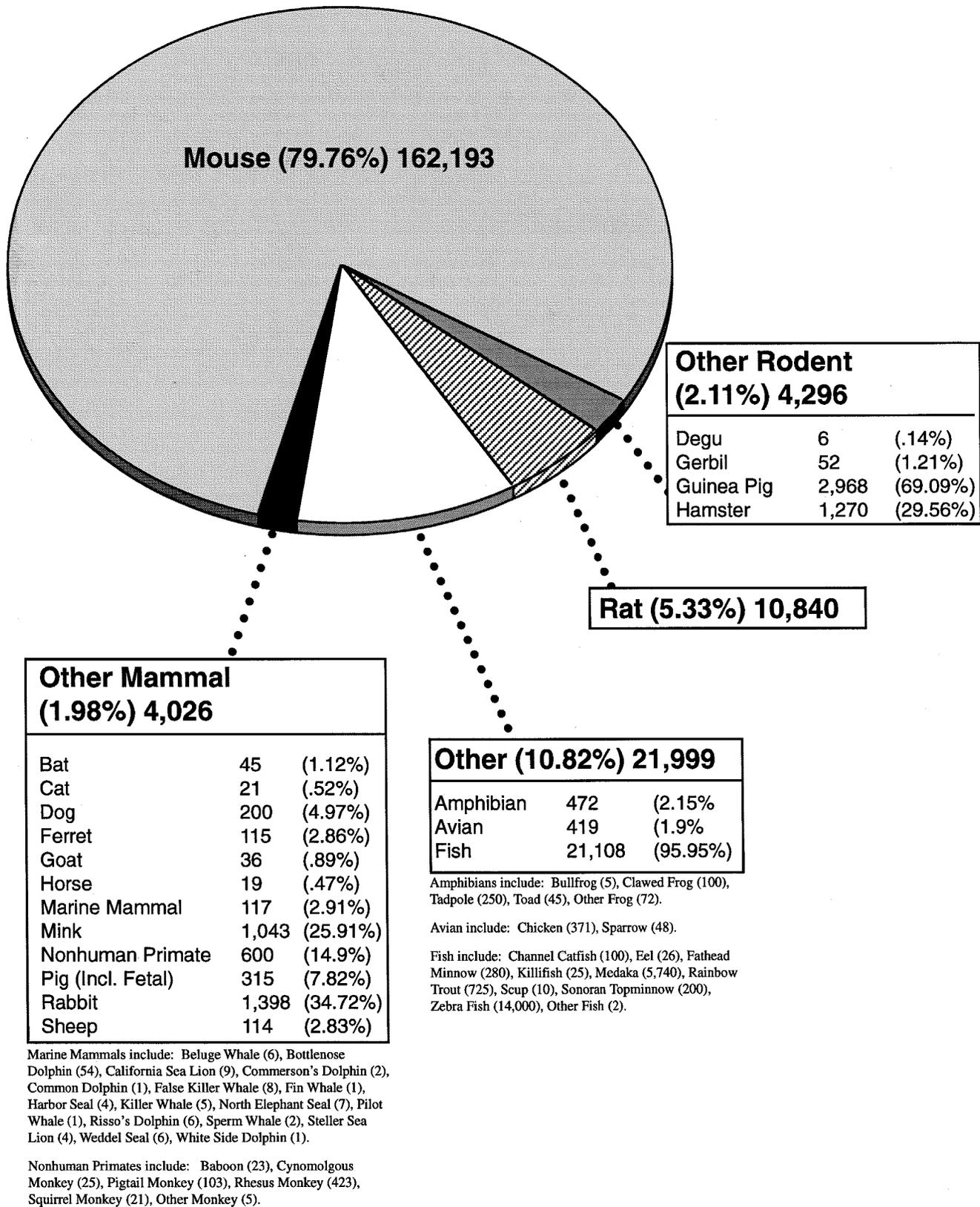


Figure V-8 Total DoD Extramural Animal Use by Species for FY95

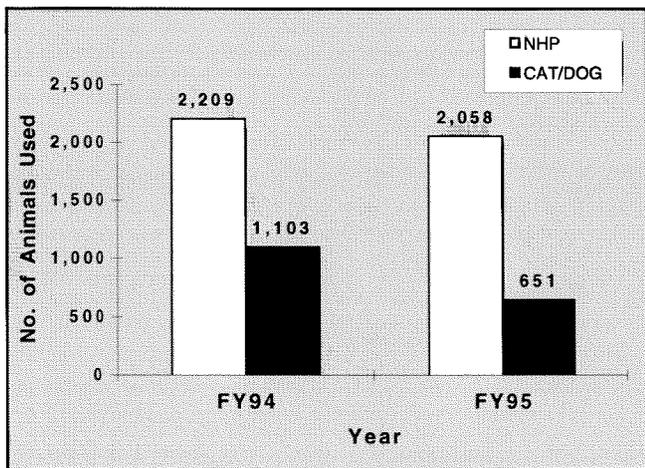
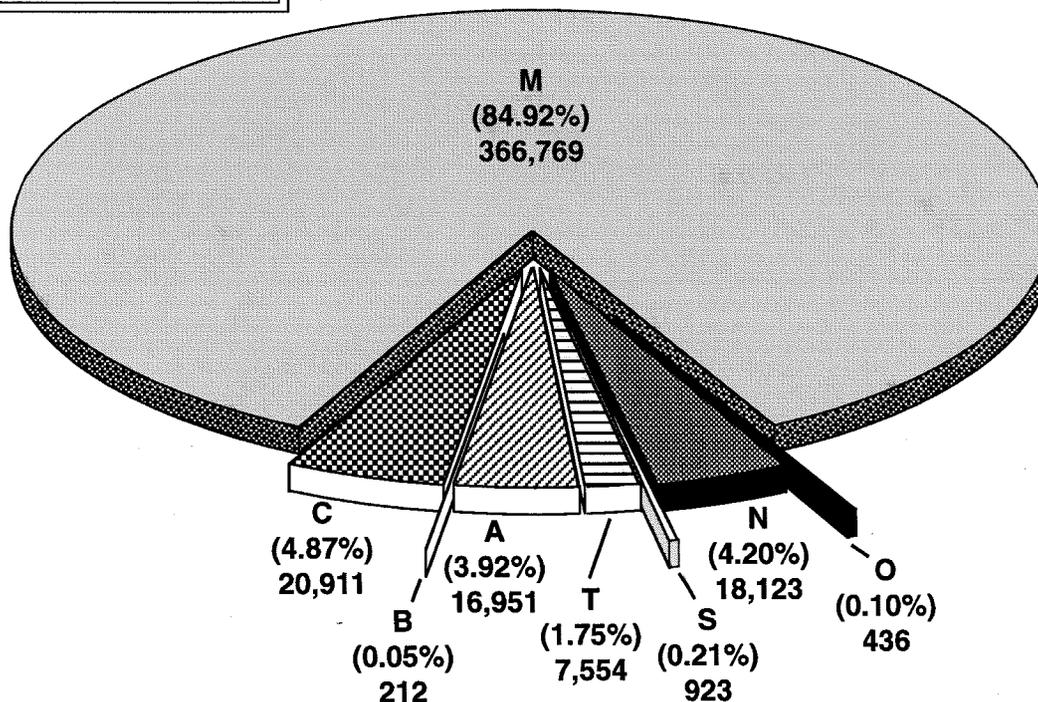


Figure V-9 Use of Nonhuman Primates, Dogs, and Cats by Year

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. 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, 90% of the animals used by the DoD in FY95 were in medical and clinical research. The majority (63%) of animals used in medical research were in the area of infectious diseases and were primarily rodents (99%) (Appendix L). 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. Ninety-one percent of the animals used in clinical research were used in clinical medicine studies.

TOTAL = 431,879



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 Total DoD Intramural and Extramural Animal Use by Category for FY95

TOTAL = 228,525

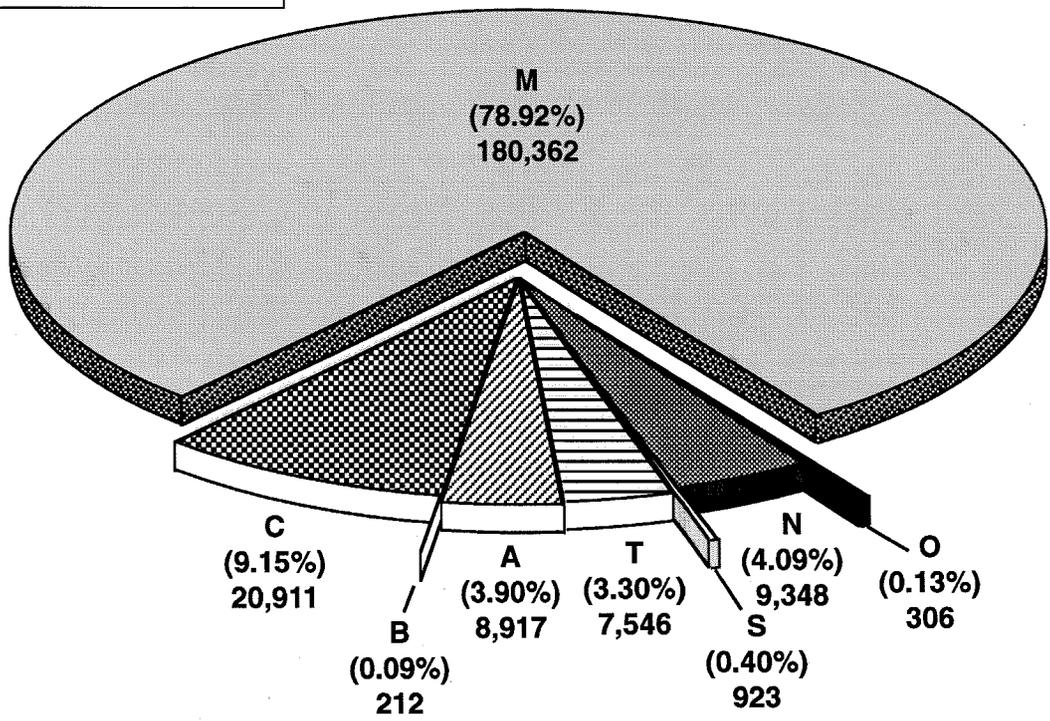


Figure V-11 Total DoD Intramural Animal Use by Category for FY95

TOTAL = 203,354

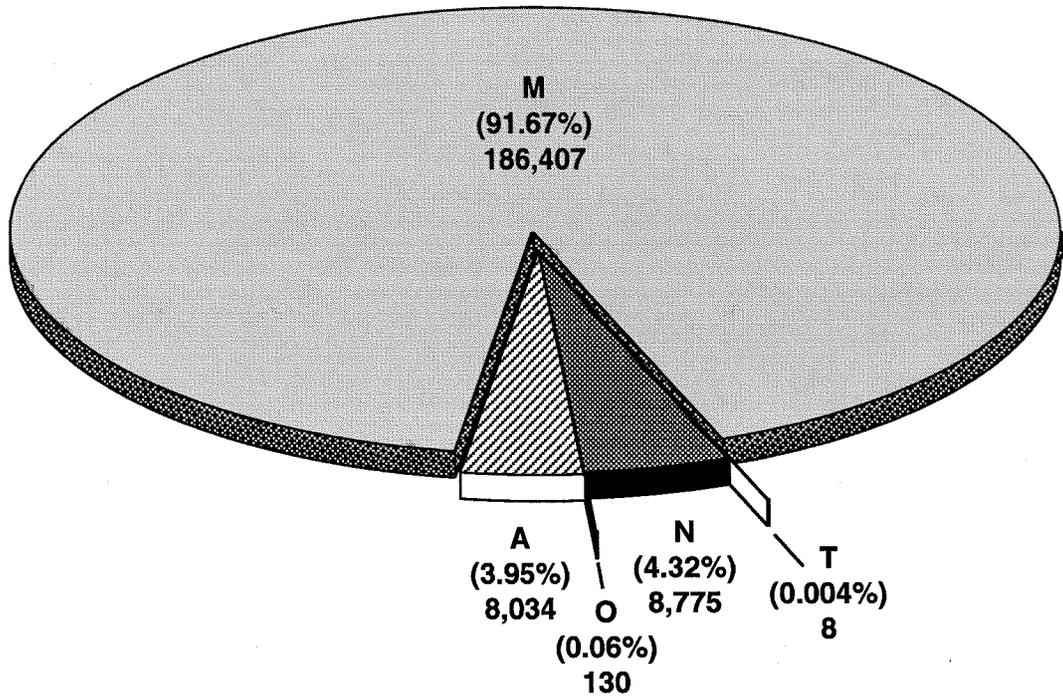


Figure V-12 Total DoD Extramural Animal Use by Category for FY95

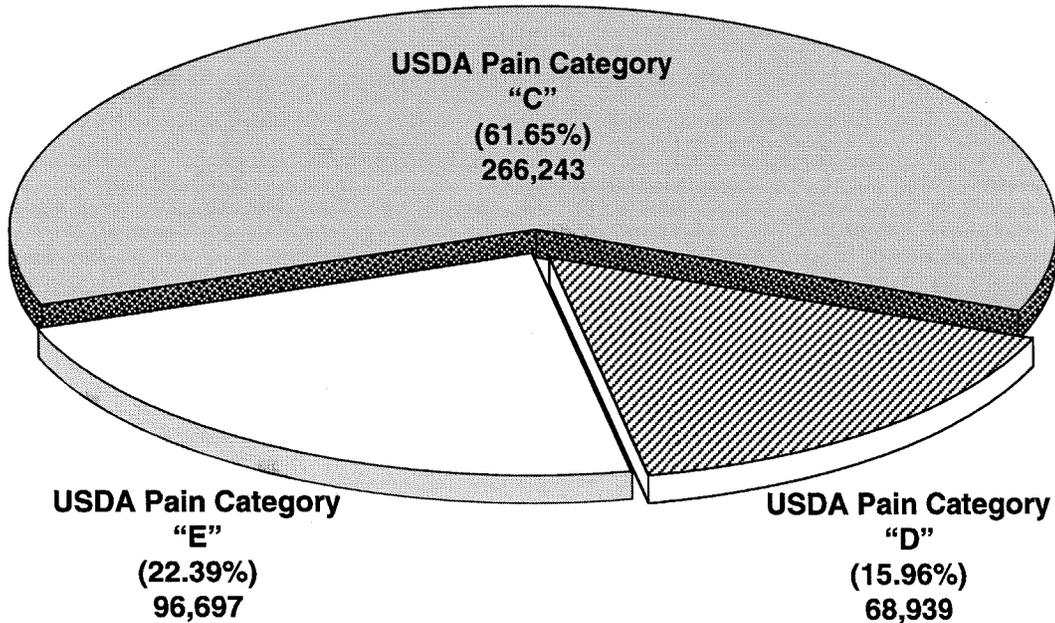
A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.
 Percentages may not add up to 100% due to rounding of calculations

Non-medical RDT&E animal use accounted for only 4% of the total animal use in FY95. The use of animals in non-medical research has steadily declined during the past 3 years. Research in the area of alternatives to the use of animals was 4% of the total animal use for FY95 and utilized primarily fish (98%). 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. In addition to the adjunct protocols focusing specifically on animal husbandry and care, in FY95 there are several ongoing actions in this area. As an example, Walter Reed Army Institute of Research has established a policy (WRAIR Policy Letter 93-27, Appendix M) that mandates consideration for environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals. No animals were used for offensive weapons testing during FY95.

V.2.5 Animal Use by USDA Pain Category

Total animal use in the DoD by USDA pain category is presented in Figure V-13, with the intramural and extramural breakouts in Figures V-14 and V-15, respectively. Most research (~78%) in the DoD was not painful to the animals involved. In the majority of the cases (62%), the animals were not exposed to or involved in any painful procedures. In 16% 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 22% of the animals used, anesthetics or analgesics were not used because they would have interfered with the results of experiments. Most (99%) of the animals used in painful experiments (where the drugs would have interfered with the results) were rats and mice. These rodents were used in medical, non-medical, and clinical research studies. There were no animals

TOTAL = 431,879



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

Figure V-13 Total DoD Intramural and Extramural Animal Use by USDA Pain Category for FY95

TOTAL = 228,525

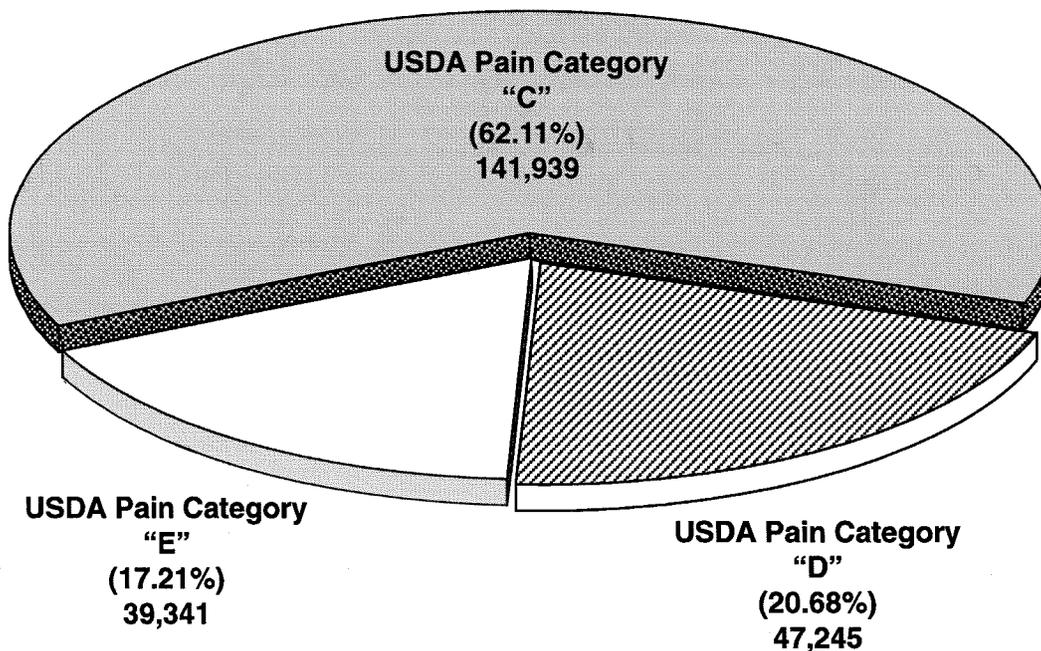


Figure V-14 Total DoD Intramural Animal Use by USDA Pain Category for FY95

TOTAL = 203,354

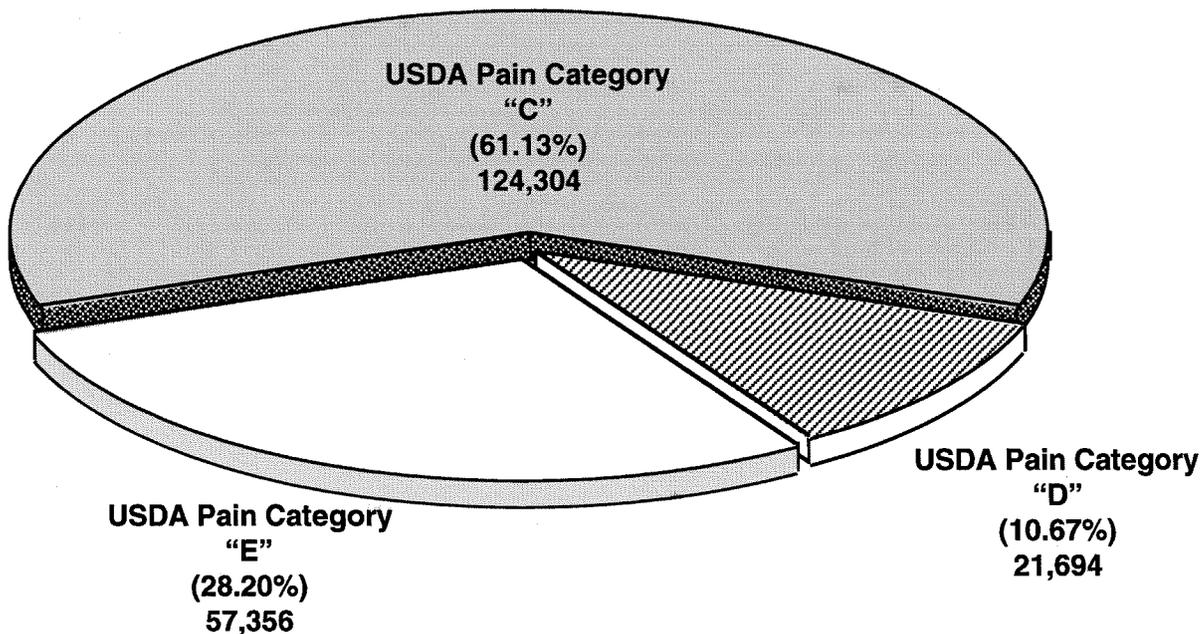


Figure V-15 Total DoD Extramural Animal Use by USDA Pain Category for FY95

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

subjected to unalleviated pain during training, secret, or alternative research studies. The DoD clearly has the 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, are of grave concern. The DoD must

provide acceptable protection against these threats and many others. The animals reported in Category E were used in the study of militarily relevant infectious disease, biowarfare defense, or chemical warfare defense efforts. This is critical research whose success is often reliant upon animal models for vaccine and efficacious counter-measure development. A large portion of these studies is driven by federal requirements, particularly those of the Food and Drug Administration. Research of this kind is not commonly done elsewhere in the government, academic, or private sectors.

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.

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, where feasible, 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 RESPONSIBILITY

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.1.1 Science and Technology Emphasis on Alternatives to Animal Subjects of Research

The Department of Defense continues to seek alternatives to animal use through an Army Science and Technology Objective (STO) initiated in FY 1993 and continuing through FY 2001 entitled *Reduced Reliance on Human and Animal Subjects of Research and Improving Experimental Conditions Using Animals*. The objectives of the program are to develop technologies to incrementally reduce future reliance on animals in research by 25% using FY91 as a base year, and to introduce a minimum of one improvement (methodology or technology) per year in experimental protocols using animals. The U.S. Army Medical Research and Materiel Command (USAMRMC) budgets approximately \$550,000 per year for this objective which is available to support alternatives to animal use research in all three services. Recent accomplishments have included incorporation of a tumor cell screening test, based on a National Institutes of Health model, for animal toxicity testing; and, the development of computer-modeled structural mutants of various toxins for screening as medical countermeasures. Efforts are in place to evaluate *in vitro* organ slice methods to replace animal testing for toxicity, and to establish and maintain advanced biomedical databases. The U.S. Army Biomedical R&D Laboratory of the USAMRMC manages a diverse research program in the development of alternative toxicity assessment methods in collaboration with the National Institute of Environmental Health Sciences, academic institutions and the private sector. Accomplishments in this program have included the development of a new non-mammalian development toxicity model, the establishment of a cooperative research and development agreement on new non-mammalian toxicity models with Colorado State University, and representing the Department of Defense on the Interagency Coordinating Committee on the Validation of Alternative Methods in toxicity testing.

The Army STO structure provides guidance, means, and high visibility to major Army technology initiatives. The Department of Army, in coordination with the Director of Defense Research and Engineering, Office of the Secretary of Defense, publishes the *Army Science and Technology Master Plan* as guidance to Army laboratories and research, development and engineering centers and to non-Army organizations supporting the Army science and technology base.

VI.1.2 Conferences and Workshops on Alternatives to Animal Use

The DoD promotes responsibility for alternatives to animal use by sponsoring formal education training programs and major meetings and conferences on the subject. In 1990, an important conference on alternatives to animal use, "DoD Initiatives in Alternatives to Animal Testing," was held at Aberdeen Proving Ground. This was followed by a 3-day symposium in 1992 entitled "Current Concepts and Approaches on Animal Test Alternatives" with 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 were published in September 1993 and are available through the Defense Technical Information Center (DTIC). In addition, in 1994 a book edited by Dr. Harry Salem entitled "Animal Test Alternatives" was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium (Appendix N).

The Department's continuing commitment to promoting responsibility for alternatives to animal use, even in an environment of constrained resources, is reflected by another such conference held on 24-26 May 1994, at Aberdeen Proving Ground entitled "Alternatives in the Assessment of Toxicity: Theory and Practice" (Appendix O). This international conference with 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States, and 45 scientific poster presentations 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"

is being published by Taylor and Francis (Appendix N). This symposium and the 1994 one were praised as a success by Dr. Martin Stephens of the Humane Society of the United States (Appendix P). A 4th Biennial International Symposium on Alternatives in the Assessment of Toxicity Issues, Progress and Opportunities was held 12-14 June 1996 at the Aberdeen Proving Ground-Edgewood Area, Maryland (Appendix O). This DoD conference was coordinated with the Scientists Center for Animal Welfare who hold their meeting 10-11 June 1996 to present Animal Welfare and Toxicology/Safety Studies: Current Issues and Trends for the Next Century. Thus a full week in Maryland was devoted to discuss Animal Welfare and Alternatives in Toxicology and Safety Studies.

DoD is also represented on the Interagency Regulatory Alternatives Group 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 National Institute of Environmental Health Sciences has established the Interagency Coordinating Committee on the Validation of Alternative Methods in response to the Revitalization Act of 1993, which also has DoD representation. 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.1.3 National Research Council, Institute of Laboratory Animal Resources, 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 Resources (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 military-specific research. The Department previously funded a 5-year ILAR grant (DAMD 17-87-G-7021) for this program and is currently in the third year of another 5-year ILAR grant (DAMD 17-93-J-3016) committing diminishing research funds to maintain this important collaboration. Annual funding for this DoD-sponsored ILAR program is in excess of \$100,000. In addition, National Research Council fellowships for conducting research in alternatives to animals are available at the U.S. Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Maryland (Appendix Q).

VI.1.4 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 Institutional Animal Care and Use Committees (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.1.5 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. Approximately 5% of the 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.1.6 Professional Veterinary Training in LAM

The individuals who are specialty trained in veterinary Laboratory Animal Medicine 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-elect and several past presidents of ACLAM, the President 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. Approximately 25% of all ACLAM boarded specialists in the U.S. received some or all of their LAM training in DoD LAM training programs.

VI.1.7 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 Library, Animal Welfare Information Center explain in detail the resources available for exploring various animal alternatives in the laboratory. The Walter Reed Army Institute of Research (WRAIR) sponsors laboratory animal workshops that provide comprehensive technical training available to all DoD personnel on animal use and related issues. Improving the technical expertise of laboratory animal technicians and investigators is a significant refinement element for the use of animals in the laboratory. These workshops are available to all DoD and National Institutes of Health laboratories. As an example, the workshop on the use of rodents is offered 14 times per year. 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.2 DoD INITIATIVES TO REPLACE, REDUCE AND REFINE THE USE OF ANIMALS

The following specific examples are a representative listing of alternative methodologies practiced in DoD facilities. They are categorized as Replacement, Reduction, and Refinement initiatives. Because of the multifaceted aspects of

many of these examples, some logically belong in more than one category. Alternative methodologies with an asterisk (*) indicate an alternative first reported in FY95 by a DoD facility or extramural contractor. Examples with a bullet (•) indicate an alternative reported in FY93 and FY94.

VI.2.1 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."

VI.2.1.A Replacement Using Biochemical or Physical Methods

- Membrane feeding systems have been developed that replace the need to feed some types of blood-feeding flies and mosquitos on rodent hosts.
- Development of Polymerase Chain Reaction (PCR) and Mammalian Cell Selection Assays for short-term genetic toxicity testing replaces animal use in carcinogenesis and mutagenesis studies.
- Efforts are ongoing to develop a PCR assay for Q-fever that could eliminate the need for the use of a mouse bioassay.
- Use of PCR for assessment of viral infections.
- Quantitating bacterial endotoxin with an *in vitro* Limulus Amebocyte test replaces *in vivo* pyrogen testing in rabbits.
- Use of predictive anthropomorphic dummies and manikins, e.g., ADAM (ejection seat reactive live load manikin) and AIRMAN (a fragment capture live fire manikin) has replaced the use of animals in these studies.

VI.2.1.B Replacement Using Computer Simulations

- Computer models to replace rhesus monkeys and baboons for toxicological studies are being developed.
- Development of computational models of dolphin echolocation (sonar) for inclusion in the development of hardware systems will replace use of animals as object detectors.
- Development for Special Forces medical training personnel of advanced computer technology using Virtual Reality, Holographic Imaging, and Telepresence Surgery techniques may replace the use of animals in Special Forces surgical training.
- Computer models are being developed for predicting carcinogenesis induced by ionizing radiation replacing the need to use animals.
- A computer model for predicting the transfer of toxic chemicals across the intestinal mucosa and into the blood stream is in development.

VI.2.1.C Replacement Using *in vitro* Cell Culture

- * Replace mice by use of bioreactor to grow large volumes of specific antibody.
- * Cell cultures are used to replace mice and rats to test inhibitors of cAMP degradation.
- * Subunits of AMPA receptors have been stably expressed in a cell. A significant portion of this study concerning drug effects of AMPA receptor physiology and ligand binding are now carried out in these cell lines.
- * Use of P450 isoenzymes to develop metabolism assays.
- * Limulus amoebocyte lysate assay has been used to test for endotoxin in vaccines.

- *In vitro* cell culture methods have been developed for passage of Hepatitis E virus eliminating use of most animals for virus propagation.
- Development of a macrophage cell line to replace animals in evaluation of cytotoxicity and genotoxicity of respirable particles is in progress.
- Development of a fish liver cell culture model for evaluating metabolism of Xenobiotic compounds replaces the use of mammalian animal models.
- Tissue culture using human gingival fibroblasts replaced the need to use rats to study the effects of Transforming Growth Factor-Beta (T6F-B) on wound healing.
- Cell cultures are being evaluated to replace mice as a host assay for detecting and identifying anthropod-borne viruses.
- Use of a rat cell line obtained from the American Type Culture Collection to study Calcium Channel Blockers and Angiotensin Converting Enzyme Inhibitors eliminates the use of pigs.
- Established cell lines from American Type Culture Collection are used in place of mice to test the effects of antibiotics on cell proliferation and inhibition of DNA synthesis as well as to test the effects of anti-progesterone chemicals on proliferation and/or inhibition and tumor cell death.
- Established cell line of macrophages from American Type Culture Collection to study the alteration of macrophage chemotactic response by oxygen replaces the use of mice and rats.
- Development of an *in vitro* hepatotoxicity screen to rank order chemicals for their ability to damage the liver will replace the use of mice and rats.
- Use of human mononuclear cells analyzed by flow cytometry to determine expression of CD69 after staphylococcal enterotoxin B

(SEB) treatment, may replace the use of mouse spleen cells.

- Cell and organ cultures to replace the rabbit for mucin-type glycoprotein in malignant breast tissue studies.
- Cell and organ cultures to replace the rat in regulated mucin gene expression in airway injury studies.
- Use of human and animal peripheral blood lymphocytes with flow cytometry to assess cytotoxicity and DNA alterations induced by sulfur mustard and its monofunctional analogue chloroethyl-ethyl sulfide replaces the use of hairless guinea pig and weanling domestic swine.
- Human cortical cell lines (HCN-1A) were used instead of rats to determine specific characteristics of sodium channels, in an effort to confirm their usefulness in studying toxins that produce their effect by sodium channel blocking.
- The HeLa Cell, a human epithelial tumor line, has been established as a useful proliferating cell model in sulphur mustard studies, replacing the use of hairless guinea pigs and weanling domestic swine.
- A terminal deoxynucleotide transferase assay was developed to measure the presence of DNA single strand breaks following sulfur mustard exposure of peripheral blood lymphocytes and human epidermal keratinocytes, replacing the use of hairless guinea pigs and weanling domestic swine.
- Human epidermal keratinocytes cultures are used as a model system to study the change in poly (ADP-ribose) polymerase (PADPRP) activity levels following sulfur mustard exposure, replacing the use of hairless guinea pigs and weanling domestic swine.
- Studies with neuroglioma cells in culture (NG108-15 cells) suggest that they are acceptable for validation of the PADPRP assay and for preliminary concentration dose response curves of sulfur mustard-induced cytotoxicity and PADPRP activity, replacing the use of hairless guinea pigs, rats, mice, and guinea pigs.
- Cells are exposed to dilute liquid sulfur mustard. At selected intervals post-exposure, samples are prepared for either biochemical or ultrastructural analyses. Analysis includes the application of specific probes (e.g., antibodies) or gel electrophoresis and electron microscopic autoradiography. Once identified, specific molecular targets are developed for use as biological markers in antivesicant drug assessment. These techniques will replace the use of hairless guinea pigs and guinea pigs.
- Living "TESTSKIN" (the commercial human skin equivalent) cellular models are used to elucidate the biochemical mechanisms responsible for sulfur mustard-induced pathology, replacing the use of hairless guinea pigs and weanling domestic swine. As the mechanisms are defined, studies of therapeutic intervention are evaluated for protection against sulfur mustard-induced pathology.
- A contract with the Cooperative Human Tissue Network provides human skin biopsies that replace the use of hairless guinea pigs and weanling domestic swine. Techniques for explant culture were developed and the specimens evaluated for histologic integrity over the first 5 days following receipt.
- Transendothelial electrical resistance and ultrastructure of cultured bovine pulmonary endothelial cells are determined after direct exposure to three edemagenic gases: phosgene, perfluoroisobutylene, and bis (trifluoromethyl) disulfide. Membrane electrical resistance is a sensitive method of determining tissue integrity and can be used to assess changes in cell-to-cell interactions that affect permeability of the endothelial barrier. These techniques replace the use of rats, guinea pigs, and sheep.

- Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are individually injected with botulinum toxin or the purified light chain of botulinum toxoid. Patch clamp recordings are used to measure capacitance changes associated with fusion of neurosecretory vesicles with the plasma membrane. Detailed examinations of membrane events during vesicle fusion are performed in the presence and absence of botulinum toxin. These techniques replace the use of mice, rats, and guinea pigs.
- Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are transfected with antisense oligonucleotides to suppress protein production from specific mRNAs. Secretion of the vesicle contents in response to potassium stimulation is then measured to assess the importance of the suppressed protein to synaptic transmission. These techniques replace the use of mice, rats, and guinea pigs.
- Study of the effects of growth factors on human fibroblasts is being conducted in cell culture media replacing the dogs and pigs utilized in previous studies.
- Development of a cell culture system to pass human breast cancer cells eliminates the need for initially passing these cells in a nude mouse model.
- Use of immortalized tissue culture systems or isolated lobster neuronal cells to investigate radiation effects and free radical damage to the nervous system at the molecular level are used to replace similar protocols using rats and guinea pigs.
- Wound-healing studies on space shuttle flights STS-45, 55 and 56 used a cell culture flight module instead of live rats.
- Development of human skin cell and animal processing plant skin models for assessing cellular mediator and tissue damage from environmental heat has replaced mammalian laboratory animal use.

VI.2.1.D Replacement with Non-Mammalian Species and Species Lower in the Phylogenetic Scale

- * Use of invertebrate sea slug (*Aplysia californica*) to study effects of chemicals on electrical properties of nerve cells to replace mammalian laboratory animals.
- * Use of guinea pigs will preclude the requirement for nonhuman primates in all but the most critical pathogenesis and protective efficacy studies.
- * Animals that are low on the phylogenetic scale (mice) are being used to determine the optimum dose schedule, route of immunization and other parameters of vaccine development. This study minimizes use of nonhuman primates.
- * Replace nonhuman primates with rats for Hepatitis E Virus bioassay.
- * Mice are used instead of nonhuman primates in newly developed test for Toxic Shock Syndrome Toxin 1.
- * Nonhuman primates are replaced with guinea pigs to test immune response to *Shigella* vaccines, and in the Leishmania Skin Test.
- Development of an aquatic bioassay using the medaka fish (*Oryzias latipes*) to assess human carcinogenic health risks replaces laboratory animal use for tumor immunodiagnosis.
- South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays and in neurotoxicology research.
- Aquatic organisms (Japanese medaka, zebrafish, bluegill, guppy) replace mammals commonly used in toxicology research.
- Rats and swine may replace cynomolgus monkeys as an alternative model for hepatitis E.

- Pigs used in emergency room and surgical resident training; and hamsters, rabbits, pigs and rats in veterinary proficiency training to replace dogs.
- Ferrets used in pediatric advanced life support courses and endotracheal intubation exercises to replace cats.
- Development of genotoxicity model using fish as an alternative to the conventional rodent model.
- Cardiopulmonary measurements previously conducted in monkeys and guinea pigs are now carried out in free-moving unrestrained rats.
- Sheep parts purchased from a processing plant are used to train dentists on periodontal surgical procedures replacing the use of live animals for training.
- Ocular researchers are using eyes purchased from local cattle processing plants for studies instead of live rabbits.
- Training programs for urology residents utilizing lasers for bladder treatments are initially performed with pig bladders purchased from a processing plant. This reduces the number of animals used for surgical training.
- Evaluation of suture patterns and angioplasty balloons on vein graft anastomosis on pigs used for surgical procedure training. Sharing of animals reduces the total number of animals used.

VI.2.1.E Replacement with Human Tissue, or Volunteers as Protocols Progress to Human Trials

- * Use of cytosensor microphysiometer which utilizes human cell lines to assess both acute and chronic toxicity. Replaces use of laboratory mammals.
- Many procedures including conjunctival impression cytology, salt and water balance and intestinal permeability, neuroendocrine assessment, nutritional support, testing of topical treatments and studies of *in vitro* activated keratinocytes in autografts in thermal injury research were previously performed in animals but have now progressed to human use protocols, eliminating the use of animals.
- Biomechanical analysis of the strength of plate fixation devices for long bone fracture repair is being performed with human cadaver bones and metal substitutes thereby replacing animal studies.

VI.2.1.F Replacement with Discarded Tissue from Other Laboratories or Food Processing Plants

- Pigs feet obtained from a local plant are used for teaching surgical suturing procedures, replacing the need for use of live animals.

VI.2.2 Reduction

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

VI.2.2.A Reduction by Use of Alternative Screening Methods to Study Efficacy in Biological Testing

- Development of a Quantitative Luminescence Imaging System for screening radiofrequency radiation biological effects in cells reduces the number of laboratory animals needed.
- Establishment of a tissue culture system to evaluate initial exposure levels of toxic substances, such as ammonia, or nitrogen and sulfur oxides, in lung and throat secretions reduces the use of animals in subsequent therapy studies.
- Development of an *in vitro* test using human peripheral blood could determine the effectiveness of toxoid in a SEB vaccine and measure the effectiveness of potential

treatments to SEB poisoning. If validated, this would significantly reduce the animals used in SEB research.

- Use of bacteria, algae, crustaceans, earthworms, flatworms, and a toxicity estimation software program functions as a screening mechanism in toxicity testing, highlighting those chemicals or materials necessitating further testing with fish or higher vertebrates. This eliminates many compounds from further testing and reduces laboratory animal use.
- Use of cell culture or molecular biology in preliminary studies of basic mechanisms of cardiovascular disease. An example is the use of an immortal cell line in molecular research on the effects of oxygen on the chemotactic response of macrophages to oxygen, reducing the need for whole animal studies.
- Development of fish (rainbow trout, zebra danio and medaka) as predictive models for epigenetic carcinogens has reduced mammalian animal use in carcinogenesis studies.
- Development and validation of fish immune responses as a biomarker to replace laboratory mammals.
- Purchase of elutriation system reduced the number of mice required for Modulation of Kupffer Cell Tumoricidal Properties by 50%.
- Toxin and toxoid preparations are titrated in a newly developed cell assay to minimize the use of animals for dose determination.
- Development of an *in vitro* test for cytoadherence by malaria-infected erythrocytes to human melanoma cells, umbilical vein cells, and endothelial cells greatly reduces the need for nonhuman primates.
- Development of a severe combined immunodeficiency disease mouse model where transplanted human liver tissue, a target for malarial sporozoite infection, cannot be rejected, permits the evaluation of potential malarial vaccine candidates in a non-monkey model.
- Development of an *in vitro* drug screening system using infected human cells to replace the mouse malaria lethality model, eliminating the need for 4,000 mice per year.
- *In vitro* drug screening, drug release kinetics, etc., result in reduction of drug candidates for numerous toxins reducing *in vivo* testing in rodent models up to 90% in some studies.
- Significant effort to develop DNA probes to detect *Orientia tsutsugamushi* in mammalian (including human) and chigger tissues should result in a 50% decrease in animal use for isolation and detection of this infectious agent.
- Development of an *in vitro* cultured human hepatoma cell line to assess radical and curative prophylactic activity of antimalarial drugs is in progress. This has the potential to reduce the number of monkeys needed for assessing antimalarial drugs and related compounds.
- *In vitro* techniques using human bone marrow cell culture to demonstrate propagation of Dengue viruses in these cells have reduced the number of monkeys needed for viral propagation by 25%.
- Development of a mosquito model using *in vitro* Dengue antigen detection techniques to pre-screen Dengue candidate vaccines should reduce the number of nonhuman primates needed for evaluation of vaccine candidates.
- Development of a reliable cell culture system for evaluating *Orientia tsutsugamushi* antibiotic resistance has reduced the need for animals for drug resistance studies by 50%.
- DNA probes have been developed to screen human *E. coli* isolates for pathogenicity. Only those positive to *in vitro* screening are tested in animals to confirm pathogenicity;

this greatly decreases the numbers of animals used.

- Use of ELISA (enzyme linked immunosorbent assay) tests as a first screen in cellular mediator (interleukin 1) studies has reduced the number of mice previously required by 90%.
- The nervous systems of invertebrate sea slugs are used to study the effect of chemical and toxic agents on the electrical properties of nerve cells. This preliminary work reduces the number of vertebrates needed for subsequent study.
- Development and use of amphibian models (*Xenopus laevis* - frog) for assessing teratogenesis assays significantly reduce mammalian animal use.
- Interlaboratory validation of the Frog Embryo Teratogenesis Assay in collaboration with NTP-NIEHS. On-going work with NTP-NIEHS to develop non-mammalian alternative methods for neurobehavioral and reproductive toxicology endpoint assessments. Collaborative work with NIEHS to use genetically engineered fish to investigate the effects of environmental contamination.

VI.2.2.B Reduction by Substitution of *in vitro* or *ex vivo* Methods

- * Numbers of mice and rats reduced to test inhibitors of cAMP degradation. Instead of 1 mouse and 1 rat per assay, 50 can be assayed from cell cultures made from 1 mouse or 1 rat.
- * Reduction in numbers of swine and goats by conducting power analysis to determine minimal numbers of animals to use in surgical studies.
- * Reduction in numbers of swine and goats in ocular studies by using same animal for both test and control eyes.

- * Reduce number of nonhuman primates and rats by focusing on selected endpoints for limited defined periods of time.
- * Reduction in use of nonhuman primates for testing SEB toxoid vaccines. Mice are used for screening, safety testing, immunogenicity evaluation, and challenge studies utilize the same nonhuman primates instead of additional animals.
- * Placing stents in both femoral arteries of rabbits allow each animal to serve as its own control.
- * Cell culture effort reduces number of rats, sparrows and chickens used in basic research.
- * Swine from other training protocols were euthanized and the eyes collected for other studies.
- * Investigators share tissues from same experimental animal (cotton rat) allowing for reduction in the number of animals utilized.
- * Candidate vaccine was tested *in vitro* in lymphocyte proliferation assay and receptor binding assay in both rodent and human cell lines.
- * Screening of antibiotics for *in vitro* activity reduces number of animals needed.
- * A nuclear magnetic resonance technique reduced the number of rats needed in a study since each animal provides data over many time points.
- * Vaccine efficacy studies in rabbit provided better predictive data for range finding and thus reduce numbers of nonhuman primates.
- Synthetic *in vitro* or *ex vivo* systems like artificial bimembrane layers, cell or tissue culture systems, and isolated diaphragm

muscle preparations replace or reduce the need for live, whole animal experiments in medical chemical defense research.

- Perfection of an *in vitro* method for growing *Plasmodium falciparum* (the most important human malaria that affects only man and certain monkey species) in human red blood cells has greatly reduced the number of nonhuman primates needed for this research.
- Development of specialized insect and vertebrate cell lines have reduced the need for intracerebral inoculation of suckling mice for the isolation of arboviruses.
- Use of transformed (immortal or self-propagating) cell cultures as an alternative to primary cell cultures that require frequent harvesting of tissues from animals.
- The use of monoclonal antibodies from hybridoma cells to replace animal-derived polyclonal antibody preparations greatly reduces animal requirements.
- Tissue culture of mouse osseous cells used as a reduction strategy for live animals to study biocompatibility of dental impression materials.
- *In vitro* techniques to orally infect mosquitoes with Dengue viruses have reduced the number of mice and monkeys needed for viral propagation by 25%.
- Development of new technology utilizing tissue slices from dead animals to assess the toxicity of selected environmental contaminants.
- Use of isolated perfused liver preparation to study the hepatotoxic effects of selected chemicals.
- Use of cultured cells for cytochrome P450 induction in vertebrate endothelium. Cells from 6 pigs represented the equivalent of approximately 100 pigs for *in vivo* studies.

- Cell cultures being developed to study mechanism of cyclic hydrocarbons and heavy metal toxicity.

VI.2.2.C Reduction by Substitution of Another Animal Species, or Human Subjects as Protocols Progress into Human Trials

- Studies have been performed to develop mouse and guinea pig models to replace the monkey as an aerosol model for botulism, staphylococcal enterotoxin B, and plague intoxication, which greatly reduces the number of monkeys needed for biological product toxicity and protective efficacy testing.
- Progression of a model of anti-malaria protective immunity into humans, where protective immunity is induced in human subjects by injected irradiated malarial sporozoites, has reduced the need for animal use in malaria research.
- Although cynomolgus monkeys are the only known model for Hepatitis E infection, rats, lesser bandicoots (rat-like animal) and swine are being evaluated as alternative models to reduce the need for monkeys.

VI.2.2.D Reduction by Substitution of Computer Simulations or Other Technologies

- * Reduction in numbers of mice, rats and guinea pigs by using radiology and assays.
- * Reduction in numbers of mice through use of computer modeling of potential peptide antigens to determine if conformation sequence is analogous to native protein.
- * Biostatistical review for research design to ensure the minimal, yet statistically significant number of animals were used.
- * Hamsters from one study were reused in another study.

- * The use of historical control data instead of animals reduces number of nonhuman primates needed in studies of vaccine efficacy.
 - * Several groups of mice were tested concomitantly so fewer control animals were needed.
 - Use of bioengineering tools to measure physiological parameters on human subjects in operational and experimental gravity tolerance environments may result in a decrease in the number of animals currently used in gravity tolerance work.
- Physiologically based pharmacokinetic modeling to predict toxicity and metabolism of trichloroethylene, vinyl chloride and their mixtures, by oral and inhalation routes reduces the use of mice and rats.
 - Development of a computer model to predict the distribution and toxic effect of candidate replacement fire extinguishing agents. This technique will reduce the use of rats.

VI.2.2.E Reduction by Sharing Animals between Research Investigations

- A research effort is aimed at developing physiologically based computer models/ algorithms to predict *in vivo* distribution, uptake, and elimination of toxic chemicals, thus reducing the need for animals.
 - Development of a computer model simulating *in vivo* absorption, distribution, metabolism, and toxic effects of nerve agents and vesicants and validated against *in vivo* pharmacokinetics data in guinea pigs for the nerve-agent soman will significantly reduce the number of animals used in nerve-agent research.
 - Training of professionals by interactive videos and innovative teaching techniques, e.g., laparoscopic instruments on synthetic sponges, reduces the use of animals.
 - Integration of mathematical modeling and aeromedical cardiovascular nonhuman primate research should reduce animal use.
 - A computer-modeling program reduces the use of sheep in blast overpressure research.
 - A computer-modeling program that identifies active sites on large molecular weight toxin molecules for intervention with therapeutic drugs is underway. This effort will substantially reduce the numbers of animals used in biotoxin studies.
 - Development of a model to understand the propagation and bioeffects of electro-
- magnetic energy should reduce the number of animals used.
 - Use of the same control animals for more than one protocol reduces the number of animals required.
 - By combining anesthesia and surgical demonstrations in goats, the numbers were reduced from eight to four.
 - Military working dogs scheduled for euthanasia are used for training labs, while under anesthesia.
 - Guinea pig tissue, required for an improved histology method for hydration and preservation of tissue morphology, is taken from guinea pigs used in other projects. Since animals are used twice, it reduces the total number of guinea pigs used per year.
 - The effect of magnesium on ventricular rate control during a trial fibrillation was studied using pigs transferred from another protocol. The re-use of swine reduced the total number of swine used per year.
 - Temperature monitoring during craniotomy procedures was carried out in conjunction with another protocol requiring swine. Re-use of swine reduced the total number of swine used per year.
 - Training in trans-septal right heart catheterization utilized sheep being euthanized as

part of another protocol. Re-use of sheep reduced the total number of sheep.

- Hearts from rats used in other experiments were utilized in studies of Growth and Characterization of Rat Cardiac Myocytes in a Capillary Cell Culture System - on Earth and in Space.
- Gastrointestinal tracts from baboons used in experiments at an independent research foundation were obtained and used in Postnatal Gastrointestinal Adaptation in Extremely Preterm Baboons with Respiratory Insufficiency: Effects of Trophic Feeds.

VI.2.3 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.

VI.2.3.A Refinement to Protocols that Reduce Pain

- * Refinement reduces pain in pigs and rabbits used for surgical training of physicians by adding long-acting local anesthetics in addition to general anesthesia.
- * Refinement in experiments on pigs assures maximal utilization using very sophisticated instrumentation.
- *Ex vivo* cardiovascular response studies (using tissues in isolated systems) of toxins eliminate potential pain and distress for animals that would be used in whole animal systems.
- Refinement of methodologies associated with the feeding of arthropod vectors (chiggers) on rodents reduces discomfort to the animals. Use of an unobtrusive barrier system to prevent escape of the chiggers eliminates the need for the attachment of a

cumbersome feeding capsule on the anesthetized animal.

- Studies performed to compare less reactogenic adjuvant regimens and alternative sites to foot pad injections in guinea pigs for evaluating hypersensitivity reactions (inflammation and swelling) from candidate Q-fever vaccines decrease potential discomfort associated with evaluation of vaccine candidates.
- Sophisticated technology such as nuclear magnetic resonance imaging is used to follow biochemical changes occurring over time in rats and other animals. This non-invasive procedure results in the use of far fewer animals and a more physiologically normal model.
- Development and evaluation of micro-encapsulated, time-released anesthetics and analgesics potentially beneficial to casualties on the battlefield have been performed. If perfected, these compounds will provide long-acting analgesia or anesthesia for animals on research projects where anesthesia or analgesia is not currently feasible.
- An evaluation of the feasibility and effectiveness of using topical analgesia (pain relief) on rabbits in Draize eye irritancy testing, and in systemic analgesia during Sereny' Testing (inflammation bioassay) on guinea pigs was performed. This provides the ability to perform a test while decreasing pain and distress without altering the outcome.
- A transdermal (applied to the skin) delivery system of analgesia to relieve pain in dogs was evaluated. It provides an extended analgesia or anesthesia for animals on research projects, and will be of benefit in human and veterinary medicine for the relief of pain.
- Use of long-acting local anesthetic in addition to general anesthesia and post-op analgesics to relieve pain in graft adhesion studies in rabbits, pericranium tissue barrier

in mandibular reconstruction studies in sheep, and pleurodesis by thoracoscopic microfibrillar collagen studies in the pig. A specially designed sling was used in the pig studies.

- In rabbit studies of repair of abdominal rectus fascia, long-acting post-op analgesics are used to reduce or eliminate pain.

VI.2.3.B Refinement to Protocols that Reduce Distress

- * Refined technique for hypothermia experiments in microswine using general anesthesia. Environmental enrichment strategies to reduce stress included mineral oil rubs and the availability of "play" objects.
- * Use of a slow release subcutaneously placed estrogen capsule avoids the need for daily intramuscular injections in rats.
- * Percutaneous techniques used for carotid artery access avoid the use of large surgical cut-down procedures on femoral arteries in rabbits.
- * Use of warming blankets and improvements in post-operative positioning of animals has improved post-operative recovery of rats and rabbits.
- * Animals were acclimated and trained to minimize pre-phlebotomy stress.
- Development of telemetric surgical procedures for implantation of sensors, allows non-stressful measurement of clinically relevant physiological parameters in non-clinical vaccine and drug efficacy studies. This not only decreases stress associated with manipulative measurements, but the radio-transmitted measurements vastly improve the quality and quantity of data available. Additionally, use of the telemetry allows physiological assessment for efficacy trials, makes intervention with analgesia more feasible, and significantly reduces the use of lethality as the primary endpoint.

- Video tapes are used for adjunct training of technicians and investigators for common animal use procedures, i.e., venipuncture, handling, and restraint.
- Novel antibody production and collection techniques in rabbits and goats with plasma collection chambers reduce potential distress associated with venipuncture procedures and reduce, and, in some cases, eliminate immunoadjuvant use.
- Use of slings for studies requiring restraint of pigs and extensive conditioning of the swine prior to initiation of the study result in a significant refinement by reducing potential distress.
- DoD facilities use social housing systems, e.g., multiple animal housing or gang caging, where feasible, which expand intraspecies interactions, and use environmental enrichment strategies that extend to many species that are not specifically mandated by animal welfare legislation. These housing strategies increase the quality of life for the animals.
- A flexible polyethylene mesh restraint device that is more comfortable and is well tolerated by rodents replaces the use of rigid restrainers previously used for maintenance of arthropod (mosquito) vectors.
- A project is underway that plays back natural nonhuman primate vocalizations and analyzes the effectiveness of this as an environmental enrichment strategy.
- A hyphema (fluid in the anterior chamber) model in rabbits has been developed using a non-invasive laser beam to open intraocular vessels and to create the hyphema instead of the standard surgical procedure previously required. This procedure eliminates post-surgical distress.
- Study endpoints are adjusted to reduce the need to proceed to death as a defined protocol objective. An example is the evaluation of the neurotoxicity of candidate therapeutic radioprotective compounds in

mice using decrements or changes in motor behavior and coordination as a definitive endpoint rather than death. Another example is using respiratory distress, rather than death, as an endpoint in the *in vivo* Study of Enhancement of Cis-Platinum Antitumor Activity by Pentoxifyllin in Nude Mice with Human Ovarian Carcinoma.

- A non-lethal model of botulism that detects intoxication by sciatic nerve paralysis in mice is under development and will be a significant refinement to the current mouse bioassay.
- By increasing quarantine time by at least a week for goats used for training, stress-related illness and deaths were decreased.
- By creating a carotid loop, the hemodynamics of simulated amniotic fluid embolism could be studied on unanesthetized sheep with minimal restraint.
- Comparison of metabolic constants for halocarbons derived from animal studies can be used to enhance the predictive value of human *in vitro* data in the risk assessment process.
- Comparison of *in vitro* results using tissues derived from the same animal to help validate the *in vitro* assay as an alternative to live animal use in toxicology research.

VI.2.3.C Refinement in Research Models and Animal Alternatives

- Professional biostatisticians are used by IACUCs to collaborate with scientists on experimental design and to review proposals in committee to ensure that only

the minimal numbers of animals needed for statistical validity are approved for use.

- Extensive use of purpose-bred, (e.g., nude mice, hairless guinea pigs) micro-biologically and genetically defined research animals yields better animal models and more meaningful and relevant research results.

VI.3 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 FY95, there was ample evidence of the DoD's aggressive pursuit of alternatives to replace, reduce and refine the use of animals, for example, USAMRMC's STO on reducing reliance on animals for research and improving experimental conditions using animals, and the development of the Frog Embryo Teratogenesis Assay toxicity test. In addition to these developmental efforts, animal use data for FY95 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 higher animals (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 now represent less than .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.

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC): A voluntary private organization that, by Fall 1995, provided accreditation for 604 institutions. AAALAC accreditation is based on the provisions of the NIH *Guide for the Care and Use of Laboratory Animals*, and is recognized by the Public Health Service.

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 assess-

ment 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. Animals covered by the act, as currently enforced, are dogs, cats, hamsters, rabbits, guinea pigs, nonhuman primates, and marine mammals.

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

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

Biological Testing: The repetitive use of a standard biological test situation or protocol employing different chemicals or different test parameters. Such test protocols are more stereotyped than those used in research, and may be more amenable to the institution of a computerized data retrieval system.

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.

Chemotactic: To attract by release of a chemical. For example, cells are attracted to a site of tissue damage by the release of chemicals by the injured cells.

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.

Draize Eye Irritancy Test: A test that involves placing a single dose of a test substance into one

eye of four to six rabbits (the other eye remains untreated) and observing its irritating effects. A promising alternative to this test is the chick embryo chorioallantoic membrane assay.

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.

ELISA (Enzyme Linked Immunosorbent Assay): An assay system that uses antibodies conjugated to enzymes. The amount of antibody attached to the molecule being analyzed can be detected by adding compounds that are cut by the enzyme releasing a colored product which can be quantified.

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, NIH *Guide for the Care and Use of Laboratory Animals*, and PHS Policy.

Institute of Laboratory Animal Resources (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 crusta-

ceans. 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.

Mutagenesis: An agent that induces chemical changes in genetic material. Chemicals, viruses, and ionizing radiation can be mutagenic. Most carcinogens are mutagens; therefore, many screening tests to detect carcinogens are designed to detect the mutagenic potential of the compound. Some mutagens are not direct acting, requiring metabolic activation in the body before they exert their mutagenic potential.

National Institutes of Health's Guide for the Care and Use of Laboratory Animals: Revised in 1985, the *Guide* details standards for animal care, maintenance, and housing. Its provisions apply to all research supported by NIH, and 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.

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 relies on the NIH *Guide for the Care and Use of Laboratory Animals*, and uses institutional committees for the assessment of programs and maintenance of records.

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.

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, however.

Sporozoite: The infectious stage of the malarial parasite that is transmitted by mosquitoes.

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

U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23, *Guide for the Care and Use of Laboratory Animals*, Revised 1985

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, 1985, "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

WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

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 1994. Department of Army, November 1993

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.

Apr 17, 95
3216.1

2. The Heads of the DoD Components shall:

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

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

c. Provide members to JTWG as required.

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

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

3. The Secretary of the Army shall:

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

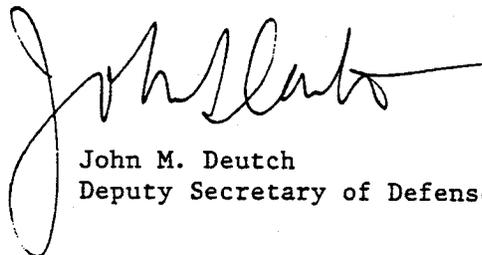
b. Designate the Commander, U.S. Army Veterinary Command/Director, DoD Veterinary Services Activity, a Field Operating Agency of the Army, Office of the Surgeon General who shall serve as a consultant to the Assistant Secretary of Defense for Health Affairs and the Director, Defense Research and Engineering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

F. EFFECTIVE DATE

This Directive is effective immediately.

Enclosures - 3

1. References
2. Definitions
3. Guidance Documents



John M. Deutch
Deputy Secretary of Defense

Apr 17, 95
3216.1 (Encl 1)

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

DEFINITION OF TERMS

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

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

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

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

Apr 17, 95
3216.1 (Encl 3)

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

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

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

Appendix B

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



OFFICE OF THE SECRETARY OF DEFENSE

WASHINGTON, D.C. 20301

10 APR 1995

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

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

References:

- (a) Title 7, United States Code, Sections 2131-2156,
The Laboratory Animal Welfare Act of 1966, PL 89-544,
as amended PL 94-279, 1976, and PL 99-198, 1985.
- (b) Review of the Use of Animals in the Department of
Defense Medical Research Facilities, Inspector General
Department of Defense, February 1994.
- (c) Review of the Use of Animals in Department of
Defense Contract Research Facilities, Inspector
General Department of Defense, August 1994.

Definition:

- (a) Animal means any dog, cat, non-human primate, or
any other live vertebrate animal which is being used
or is intended for use for research, training, testing,
or experimentation purposes. For this Policy Guidance,
it includes birds, rats of the genus Rattus and mice of
the genus Mus bred for use in research, training,
testing or experimentation purposes. The term excludes
animals used for ceremonial or recreational purposes,
military working animals, and animals intended for use
as livestock and poultry as food or fiber; or,
livestock or poultry used or intended for use for
improving animal nutrition, breeding, management, or
production efficiency, or for improving the quality of
food or fiber.
- (b) DoD-Sponsored programs means any study, proposal,
or design for animal experimentation or demonstration
in Research Development, Test, and Evaluation (RDT&E),
clinical investigation, or instructional program
conducted or funded by grant, award, loan, contract, or
cooperative research and development agreement (CRADA).

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

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

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

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

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

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

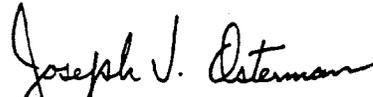
b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for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

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

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



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



Joseph V. Osterman
Director, Environmental
and Life Sciences

Attachments:

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

Appendix C

DoD Standard IACUC Protocol Format Instructions

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

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

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

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

(Signature Required)

(Principal Investigator)

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

(Signature Required)

(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN: (Example) The attending/consulting veterinarian has reviewed the protocol and was consulted in the planning of procedures that require veterinary input, i.e., an unalleviated pain procedure. In addition, the veterinarian/veterinary medicine department has assisted with coordination for veterinary support to the protocol. (No response is required to the title paragraph of this section)

(Signature Required)

(Attending/Consulting Veterinarian)

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

(Statistician)

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

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

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

II. BACKGROUND:

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

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

1. **Literature Source(s) Searched:**

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

3. **Key Words of Search:**

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

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

IV. MILITARY RELEVANCE: With regards to military needs and mission requirements, this paragraph should provide a brief and succinct military justification for the research. If applicable state the Science and Technology Objective (STO) that this work supports.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures: Provide a "complete description of the proposed use of animals." This section should succinctly outline the formal scientific plan and direction for experimentation. If several experiments or sequential studies

are to be included in the protocol, description of the experimental design for each separate experiment should be contained in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. However, **a clearly understandable description of the numbers of animals and their distribution into experimental groups is essential.** The number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. If too few animals are requested and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development, etc. If the design is complex, a summary table or flow chart showing the distribution of animals by experimental group should be included. **The total number of animals required for the study is listed in section V.B.4.** It is critical that reviewers of this protocol are able to follow your reasoning and calculations for the number of animals required, and can verify that the experimental design clearly supports the number of animals requested.

1. Experiment 1:
2. Experiment 2: (etc.)

B. Laboratory Animals Required and Justification:

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

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

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

a. Genus & Species:

b. Strain/Stock: If inbred or specialized animals are required, please use proper terminology.

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

d. Age:

e. Weight:

f. Sex:

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

h. Other:

4. Total Number of Animals Required:

(a) mice	320
(b) guinea pigs	175

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

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

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

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

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

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

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

1. Pain: The law defines a painful procedure as one that would "reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." **If a procedure involves pain or distress, the P.I. must consult with the attending veterinarian.** Respond N\A if the animals will experience "no pain or distress."

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

This information is reported by the organization to the USDA on USDA Form VS 18-23. **The P.I. or primary user should estimate the number of animals that will be counted in each pain category.** There are many situations where there are animals in more than one category, i.e., control animals. If more than one species is requested in the proposal, reflect those animals in a duplicate table in this paragraph. **The total numbers reflected in these three categories should add up to the number and percent of animals requested for the entire protocol in para V.B.4.**

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

Studies involving no pain or distress beyond that expected on a momentary nature such as would occur with an injection, a deep palpation, grooming activities, etc.

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

Procedures wherein anesthesia or analgesia will be administered to avoid or alleviate pain or distress. General anesthesia given for

surgical preparations, or the use of analgesia or anti-inflammatories would be examples for this category.

(3) Unalleviated Pain or Distress

_____ (#) _____ % (Column E)

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

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

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

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

c. Alternatives to Painful Procedures:

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

(2) **Date of Search:**

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

(4) **Results of Search:** Provide a narrative description of the results of the alternatives literature search. "Research facilities will be held responsible, if it is subsequently determined that an alternative to a painful procedure was available to accomplish the objectives of the proposed experiment." The Animal Welfare Act specifically states that the "P.I. must provide a narrative description of the methods and sources, e.g., the Animal Welfare Information Center, MEDLINE, LIFE SCIENCES ABSTRACTS, AGRICOLA, AND BIOSIS that he\she used to determine that alternatives to the painful procedure were not available." It is a requirement to perform the alternatives literature search and painful procedure justification even when animals are placed in the alleviated pain category (column D).

d. Painful Procedure Justification: Procedures

causing more than transient or slight pain that are unalleviated, must be justified on a scientific basis in writing by the P.I. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized. This paragraph must be completed if there are any animals listed in either the alleviated (column D) or the unalleviated pain or distress (column E) category in para V.C.1. **The P.I. must consult with the attending veterinarian or his or her designee in the planning of both alleviated and unalleviated painful procedures, and state it here.**

2. **Prolonged Restraint:** Describe and justify in detail any prolonged restraint (greater than twelve hours) intended for use during the study, e.g., primate chairs, restraint boards, metabolism cages, etc. Also describe habituation procedures for the prolonged restraint. This section is not intended for short-term actions such as rabbit restraint for bleeding, etc. If there is prolonged restraint involved, who will be restraining the animals, and for how long?

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

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

b. **Pre- and Postoperative Provisions:** Detail the provisions for both pre- and postoperative care, including provisions for post-surgical observations. Also include the provider of that care, and the location for the postoperative care.

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

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

(1) Procedures:

(2) Scientific Justification:

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

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

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

c. Animal Identification: Microchip, tattoo, ear tags, cage cards, etc.

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

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

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

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

7. Euthanasia: Explain the plan for euthanasia of the animals at the completion of the study and who will perform the procedure. The AWA defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent

death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current AVMA guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. Exceptions must be scientifically justified by the P.I. in writing. The attending veterinarian will assist in selecting the best method for euthanasia if requested.

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

1. Husbandry Considerations: The law specifically states that animal housing and living conditions must be appropriate to their species, and contribute to their health and comfort. Describe husbandry or refer to SOP. If known, list the location the animals will be routinely housed and the length of housing requirement. Personnel in the animal care unit should be able assist P.I.s in the preparation of the protocol sections dealing with animal care issues.

a. Study Room: If stay exceeds 12 hours.

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

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

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

a. Dogs: Do you have any reason to restrict activity programs for dogs on this protocol that might be implemented by the animal care unit to comply with federal welfare regulations. If yes, justify.

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

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

F. Investigator & Technician Qualifications/Training: List those animal procedures or manipulations described in the protocol that will be performed by each investigator or technician, and their training or qualifications to perform these procedures. Personnel conducting the "hands-on" animal procedures described in the protocol must be identified and appropriately trained and qualified to perform that procedure. **This is NOT questioning the P.I.'s PROFESSIONAL qualifications to conduct the research, but rather a requirement that personnel actually performing the research animal manipulations are technically competent, and thus are not inflicting unnecessary pain, distress, or injury to an experimental animal due to inexperience or improper technique.** Contact your attending veterinarian for assistance with this requirement.

VI. Biohazard/Safety: Provide a list of any potential biohazards associated with this proposal, e.g., viral agents, toxins, radioisotopes, oncogenic viruses, chemical carcinogens, etc. Explain any safety precautions or programs designed to protect personnel from biohazards, and any surveillance procedures in place to monitor potential exposures.

(Start new page here)

VII. ASSURANCES: The law specifically requires several written assurances from the P.I. It states that "research facilities will be held responsible if it is subsequently determined that an experiment is unnecessarily duplicative, and that a good faith review of available sources would have indicated as much."

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

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the IACUC.

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

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

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

E. Training: I verify that the personnel performing the

animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Signature Required)

(Primary Investigator)

G. Painful Procedures: (Include only if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers.** I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

(Signature Required)

(Primary Investigator)

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

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

B. Pathology Addendum: Optional information

C. Pain Scoring Guidelines:

D. Adjuvant Policy:

PROTOCOL COVER SHEET

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

(Signature Required)

(Principal Investigator)

SCIENTIFIC REVIEW:

(Signature Required)

(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN:

(Signature Required)

(Attending/Consulting Veterinarian)

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

(Statistician)

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

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

- I. NON-TECHNICAL SYNOPSIS:
- II. BACKGROUND:
 - A. Background:
 - B. Literature Search:
 - 1. Literature Source(s) Searched:
 - 2. Date and Number of Search:
 - 3. Key Words of Search:
 - 4. Results of Search:
- III. OBJECTIVE\HYPOTHESIS:
- IV. MILITARY RELEVANCE:
- V. MATERIALS AND METHODS:
 - A. Experimental Design and General Procedures:
 - B. Laboratory Animals Required and Justification:
 - 1. Non-animal Alternatives Considered:
 - 2. Animal Model and Species Justification:
 - 3. Laboratory Animals:
 - a. Genus & Species:
 - b. Strain/Stock:
 - c. Source/Vendor:
 - d. Age:
 - e. Weight:
 - f. Sex:
 - g. Special Considerations:
 - h. Other:
 - 4. Total Number of Animals Required:
 - 5. Refinement, Reduction, Replacement:
 - a. Refinement:
 - b. Reduction:
 - c. Replacement:
 - C. Technical Methods:
 - 1. Pain:
 - a. USDA (Form 18-3) Pain category:
 - (1) No Pain _____ (#) _____% (Column C)
 - (2) Alleviated Pain _____ (#) _____% (Column D)
 - (3) Unalleviated Pain or Distress
_____ (#) _____% (Column E)
 - b. Pain Alleviation:
 - (1) Anesthesia/Analgesia/Tranquilization:
 - (2) Paralytics:
 - c. Alternatives to Painful Procedures:
 - (1) Source(s) Searched:
 - (2) Date of Search:
 - (3) Key Words of Search:
 - (4) Results of Search:
 - d. Painful Procedure Justification:

2. Prolonged Restraint:
3. Surgery:
 - a. Procedure:
 - b. Pre- and Postoperative Provisions:
 - c. Location:
 - d. Multiple Survival Surgery Procedures:
 - (1) Procedures:
 - (2) Scientific Justification:
4. Animal Manipulations:
 - a. Injections:
 - b. Biosamples:
 - c. Animal Identification:
 - d. Behavioral Studies:
 - e. Other procedures:
5. Adjuvants:
6. Study Endpoint:
7. Euthanasia:
- D. Veterinary Care:
 1. Husbandry Considerations:
 - a. Study Room:
 - b. Special Husbandry Provisions:
 2. Attending Veterinary Care:
 3. Enrichment Strategy:
 - a. Dogs:
 - b. Nonhuman Primates:
- E. Data Analysis:
- F. Investigator & Technician Qualifications/Training:
- VI. Biohazard/Safety:

(Start new page here)

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

A. **Animal Use:** The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the IACUC.

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

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

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

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

F. **Responsibility:** I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Signature Required)

(Primary Investigator)

G. **Painful Procedures:** (Include above if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

(Signature Required)

(Primary Investigator)

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

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

B. Pathology Addendum: Optional information

C. Pain Scoring Guidelines:

D. Adjuvant Policy:

Appendix D

DoD Semiannual Program Review and Facility Inspection Checklist

DOD SEMIANNUAL PROGRAM REVIEW/FACILITY INSPECTION CHECKLIST-MANDATORY

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

ORGANIZATION: _____ DATE OF REVIEW: _____

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

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

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

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

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

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

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

-OPTIONAL-

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

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

B. Institutional Policies

1. Monitoring the Care and Use of Animals

a. Institutional Animal Care and Use Committee

1) Composition- New DoD Directive states the minimum number for IACUC membership is 5. New DoD policy states requires those IACUCS with only one non-affiliated member the IACUC to also appoint an additional alternate non-affiliated member. New DoD policy states specific training requirements for non-affiliated IACUC members (8 hours).

2) Protocol review procedures- New DoD Directive and policies require use of DoD standard protocol format. New requirements include documentation of literature searches for DTIC, FEDRIP and other searches as required.

3) Review of programs for Care and Use of Animals- New DoD policy encourages Commanders/Directors/CEO's of DoD laboratories to invest in training at all levels for those that use animals.

b. USDA Report

2. Veterinary Care

a. Intensity -

b. Responsibilities of the Veterinarian(s) -

c. Involvement in monitoring the care of animals -

d. Involvement in monitoring use of animals -

3. Personnel Qualifications

a. Animal resource Professional/Management/ Supervisory Personnel -

b. Animal Care Personnel -

c. Research Staff -

d. Use of Hazardous Agents -

4. Personnel Hygiene

a. Work clothing provided -

b. Laundering of work clothing -

c. Shower and change facilities -

d. Eating, drinking, and smoking policies -

e. Eating, drinking, and smoking facilities -

5. Occupational Health and Safety Program

a. Content of program -

b. Program oversight -

c. Participation by staff -

d. Training on zoonosis and personal hygiene -

6. Experimentation involving Hazardous Agents

7. Animal Restraint -

8. Multiple Major Surgical Procedures -

C. Laboratory Animal Husbandry

1. Housing

a. Caging and pens -

DoD Semiannual Program Review/Facility Inspection

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

DoD Semiannual Program Review/Facility Inspection

- 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 -

DoD Semiannual Program Review/Facility Inspection

e) Postoperative care -

3. Animal Rooms

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

4. Other Features

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

5. Miscellaneous Animal Care and Use Equipment

F. Special Considerations

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

G. Study Areas Visited -

H. Laboratories Visited -

DoD Semiannual Program Review/Facility Inspection

-OPTIONAL-

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

Building _____

=====

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

=====

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

=====

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

=====

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

=====

GENERAL COMMENTS:

Appendix E

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

Appendix E

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 F

Nonaffiliated IACUC Members Professions

Appendix F

Nonaffiliated IACUC Members Professions

Accountant
Administrative Assistant
Attorney
Biologist
Chaplain
Chemist
Clinical Health Worker
Communications Expert
Dentist
Engineer
Health Educator
Health Services Administrator
Homemaker
Information Systems Specialist
Lab Scientist
Law Enforcement
Manpower Management Analyst
Medical Records Librarian
Microbiologist
Nurse
Personnel Consultant
Physician
Public Affairs Officer
Teacher
Veterinarian
Veterinary Technologist/Animal Services

Appendix G

Dissemination of Information on Animal Care and Use

Appendix G

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
- Video tapes
- Investigators' handbooks
- Directed discussions at IACUC meetings
- Newsletters such as Scientists Center for Animal Welfare

Appendix H

IACUC Training and Information

Appendix H

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:

1. Humane Care and Ethics Issues Dealing With Animal Use (This block should be NLT 4 hours long)

2. Regulatory Responsibilities and Protocol Review Techniques (This block should be NLT 4 hours long)

3. Facility Familiarization Tour

4. Basic Husbandry and Techniques of Laboratory Animals

5. Documentation of Training

Resources:

- 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)
- 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)
- attending veterinarian, facility manager, IACUC members
- LATA video tapes and script
- ACLAM slide sets with audio cassettes
- USAMRIID slide set
- 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
- Video tapes
- 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 I

Journals with DoD Animal Research Publications

Appendix I

Journals with DoD Animal Research Publications

Accident, Analysis, & Prevention
Acta Tropica
American Journal of Cardiovascular Pathology
American Journal of Dermatopathology
American Journal of Otolaryngology
American Journal of Respiratory Critical Care Medicine
American Journal of Tropical Medicine Hygiene
American Journal of Veterinary Research
American Journal of Physiology
Analytical Letters
Archives of Oral Biology
Archives of Toxicology
Aviation, Space, and Environmental Medicine
Behavioral and Neural Biology
Biochemistry
Biotechnology
Blood
Brain Research
Brain Research Bulletin
Burns
Chemical Biological Interactions
Chemical Research in Toxicology
Chest
Chirality
Circulation
Clinical Immunotherapy
Clinical Pharmacology & Therapeutics
Clinical Research
Contemporary Topics in Laboratory Animal Science
Diabetes
Drug and Chemical Toxicology
Drug Development Research
Endocrinology
Environmental Toxicology and Chemistry
Epilepsy Research
European Journal of Immunology
Experimental Cell Research
Experimental Hematology
Experimental Parasitology
FASEB Journal
Federal Practitioner
Fundamental Applied Toxicology
Gastroenterology
Hemoglobin
Human and Experimental Toxicology
Immunity

Infection and Immunity
Inflammation
Inhalation Toxicology
International Journal of Immunopharmacology
International Journal of Radiation Biology
International Journal of Sports Medicine
Investigative Ophthalmology and Visual Science
Journal of Acoustical Society of America
Journal of American Academy of Dermatology
Journal of American College of Surgeons
Journal of American Colleges of Cardiology
Journal of American Medical Association (JAMA)
Journal of Analytical Toxicology
Journal of Applied Toxicology
Journal of Burn Care and Rehabilitation
Journal of Chromatography
Journal of Clinical Microbiology
Journal of Clinical Periodontology
Journal of Cutaneous Pathology
Journal of Dental Research
Journal of Diarrheal Research
Journal of Experimental Medicine
Journal of Immunology
Journal of Infectious Disease
Journal of Investigative Surgery
Journal of Medical Entomology
Journal of Neuroscience Methods
Journal of Nutrition
Journal of Pharmacological and Toxicological Methods
Journal of Pharmacology and Experimental Therapeutics
Journal of Pharmacy and Pharmacology
Journal of Physiology
Journal of Submicroscopic Cytology and Pathology
Journal of the American Mosquito Control Association
Journal of the American Veterinary Medicine Association
Journal of the Experimental Analysis of Behavior
Journal of Trauma
Laboratory Animals
Laboratory Animal Science
Lymphokine and Cytokine Research
Management of Wilderness and Environmental Emergencies
Medical Veterinary Entomology
Microbiology
Molecular and Biochemical Parasitology
Molecular Microbiology
Neuropharmacology
Neurotoxicology
Oral Microbiology and Immunology
Parasite Immunology
Parasitology Research
Pediatric Pulmonology
Pediatric Research
Pharmacology

Pharmacology, Biochemistry and Behavior
Physiology and Behavior
Proceedings of the Society of Experimental Biology and Medicine
Proceedings of the National Academy of Science
Radiation Research
Shock
Southern Medical Journal
Thrombosis Haemostasis
Tissue and Cell
Toxicologist
Toxicology
Toxicology and Applied Pharmacology
Toxicology Methods
Toxicon
Tropical Geographical Medicine
Undersea & Hyperbaric Medicine
Vaccine
Veterinary Pathology

Appendix J

Status of AAALAC Accreditation of DoD Facilities

Appendix J

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

- Dwight David Eisenhower Medical Center, Fort Gordon, GA

I.3 U.S. Navy:

- Naval Dental Research Institute, Naval Training Center, Great Lakes, IL
- Naval Medical Center, Clinical Investigation Program, San Diego, CA
- Naval Medical Center, Clinical Investigation and Research, Portsmouth, VA
- Naval Medical Research Institute, Bethesda, MD
- Naval Command, Control and Ocean Surveillance Center, San Diego, CA

I.4 U.S. Air Force:

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

II U.S. DoD Programs Actively Involved in the AAALAC Process:

II.1 U.S. Army:

- U.S. Army Institute of Surgical Research, Fort Sam Houston, TX, has applied for AAALAC accreditation
- U.S. Army Dugway Proving Ground, UT has applied for AAALAC accreditation

II.2 U.S. Air Force Programs:

- U.S. Air Force Academy has applied for AAALAC accreditation

III 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

IV Overseas DoD Program Actively Involved in the AAALAC Process:

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

Appendix K

Animal Use Categories

Appendix K

Animal Use Categories

MEDICAL (M)

M1: Military Dentistry

Includes studies in the areas of:

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

M2: Infectious Diseases

Includes studies in the areas of:

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

M3: Medical Chemical Defense

Includes studies in the development of:

- medical countermeasures for vesicant agents

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

M4: Medical Biological Defense

Includes studies in the development of medical countermeasures for:

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

M5: Human Systems Technology

Includes studies on:

- bioeffects of lasers
- laser impacts on performance
- treatment of laser-induced injury
- development of predictive models for a non-auditory exposure standard for blast 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
- occupational health
- vision
- free electron laser

NON-MEDICAL (N)

N1: Physical Protection

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

- developing hearing protection criteria
- mechanisms of and protection from military acoustic hazards

- ocular effects and performance of eye protective devices

N2: Physical Detection

Includes studies in the development of:

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

N3: Offensive Weapons Testing

No studies performed in this category

N4: Other Non-Medical RDT&E

Includes studies in the areas of:

- environmental toxicology
- basic biological research
- human systems technology
- acoustics research
- chronobiology
- robotics
- pressure biology
- physiology

CLINICAL INVESTIGATIONS (C):

C1: Clinical Medicine

Research conducted includes a wide variety of clinical medical diseases/conditions which were not necessarily unique to the military. Includes studies in the areas of:

- burn treatment
- prophylaxis against toxic chemicals
- wound healing
- preservation of tissue sample morphology
- differentiation of brain tumors
- substances promoting repair of sound-sensing cells
- regulation of tracheal mucin secretion by retinoic acid

- breast cancer research
- mechanisms and treatment of renal pathophysiology
- effects of tumor necrosis factor on gonadotrophic activity
- treatment of immune-mediated hearing loss
- mechanisms of lung growth and compensation following injury
- testing of hepatitis-E vaccines

C2: Clinical Surgery

Includes studies in the areas of:

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

C3: Other Clinical Investigations

None in FY95

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

Includes training/instruction in the areas of:

- medical fellows/residents research projects
- veterinary fellows/residents research projects

ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A):

A1: Adjuncts to Animal Use Research

Addresses those studies and uses which focused specifically on animal husbandry and care issues, and not directly on human medical, non-medical, or training issues.

A2: Alternatives to Animal Investigation

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

A3: Other Alternatives/Adjuncts

None in FY95

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. The total numbers of animals in this category cannot be reported. However, the total number is less than 0.1% of all animals used by the DoD in FY95.

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

Appendix L

Summary of Animal Use Data by Category

Appendix L

Summary of Animal Use Data by Category

MILITARY DENTISTRY		
Category	Species	Animals Used
M1	MOUSE	60
M1	RABBIT	18
M1	RAT	14
M1	RICE RAT	64
MILITARY DENTISTRY TOTAL		156

INFECTIOUS DISEASES

Category	Species	Animals Used
M2	AFRICAN GREEN MONKEY	6
M2	ARMANIAN HAMSTER	5
M2	BANDICOOT RAT	7
M2	BIRD	8
M2	CHICKEN	141
M2	CYNOMOLGOUS MONKEY	19
M2	DOG	114
M2	FERRET	12
M2	GERBIL	31
M2	GOAT	1
M2	GOOSE	4
M2	GUINEA PIG	1,501
M2	HAMSTER	1,837
M2	HORSE	19
M2	MASTOMY	250
M2	MONKEY	85
M2	MOUSE	223,400
M2	PIG	204
M2	PIGTAIL MONKEY	101
M2	RABBIT	1,026
M2	RAT	2,140
M2	RHESUS MONKEY	464
M2	ROBIN	7
M2	SEA SLUG	26
M2	SHEEP	115
M2	SQUIRREL MONKEY	15
M2	STARLING	7
INFECTIOUS DISEASES TOTAL		231,545

MEDICAL CHEMICAL DEFENSE

Category	Species	Animals Used
M3	BULLFROG	5
M3	DOG	21
M3	EEL	1
M3	FROG	60
M3	GUINEA PIG	1,570
M3	MOUSE	5,080
M3	PIG	72
M3	RABBIT	274
M3	RAT	2,498
M3	RHESUS MONKEY	127
M3	TADPOLE	250
M3	WEANLING PIG	40
MEDICAL CHEMICAL DEFENSE TOTAL		9,998

MEDICAL BIOLOGICAL DEFENSE

Category	Species	Animals Used
M4	AFRICAN GREEN MONKEY	8
M4	BURRO	1
M4	COW	0
M4	CYNOMOLGOUS MONKEY	137
M4	GOAT	15
M4	GOOSE	15
M4	GUINEA PIG	896
M4	HAMSTER	337
M4	HORSE	77
M4	MOUSE	44,003
M4	RABBIT	323
M4	RAT	1,432
M4	RHESUS MONKEY	210
M4	SHEEP	84
MEDICAL BIOLOGICAL DEFENSE TOTAL		47,538

HUMAN SYSTEMS TECHNOLOGY

Category	Species	Animals Used
M5	AFRICAN TOAD	5
M5	CAT	3
M5	CHINCHILLA	19
M5	DOG	52
M5	FISH	0
M5	FROG	171
M5	GUINEA PIG	73
M5	HAMSTER	280
M5	MEDAKA FISH	1,850
M5	MOUSE	594
M5	PIG	198
M5	PIGEON	23
M5	RABBIT	103
M5	RAINBOW TROUT	20
M5	RAT	3,111
M5	RHESUS MONKEY	69
M5	SEA SLUG	25
M5	SHEEP	10
M5	TOAD	45
M5	ZEBRA FISH	10,000
HUMAN SYSTEMS TECHNOLOGY TOTAL		16,651

COMBAT CASUALTY CARE

Category	Species	Animals Used
M6	BABOON	23
M6	BONNET MONKEY	2
M6	CAT	24
M6	DOG	50
M6	GOAT	4
M6	GUINEA PIG	131
M6	HAMSTER	180
M6	MOUSE	17,204
M6	PIG	405
M6	RABBIT	468
M6	RAT	6,772
M6	SHEEP	96
COMBAT CASUALTY CARE TOTAL		25,359

Appendix M

**Walter Reed Army Institute Policy 93-27 - Laboratory Animals
Environmental Enrichment Program**



DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, D.C. 20307-5100



IN REPLY REFER TO:

SGRD-UWN (310-2d)

13 DEC 1991

MEMORANDUM FOR SEE DISTRIBUTION

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal
Environmental Enrichment Program

1. NONHUMAN PRIMATES

a. Applicable Division of Veterinary Medicine Standard
Operating Procedures:

SOP-DAR-760 - Environmental Enrichment - General
SOP-DAR-761 - Environmental Enrichment of Nonhuman Primates

b. PSYCHOLOGICAL WELL-BEING:

(1) Social Grouping. The Division of Veterinary
Medicine (DVM) has, as a goal, the pairing or grouping of as many
nonhuman primates (NHP) as is feasible. While recognizing that
group housing of most nonhuman primates is the ideal, the DVM is
constrained by space and personnel limitations. Even without
these constraints, aggressive behavior exhibited by some NHPs
precludes the pairing or grouping with conspecifics.

(a) Thirty-two (32) socialization units housing
rhesus monkeys in compatible pairs are in use. An additional
eight (8) are reserved for bi-weekly cage changeouts.

1) Animals selected for pairing are chosen
based on mutual compatibility.

2) Selection criteria for pairing are as
follows:

- a) Young animals
- b) Animals with behavioral problems
such as self-mutilation or
excessive grooming
- c) Adult females
- d) Younger animals paired with an
adult male
- e) Adult males (after pulpectomy of
canine teeth)

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

(3) An environmental enrichment log is maintained by the Division of Veterinary Medicine at Building 40 and the Gillette Building. Veterinary personnel record the details of daily enrichment activities such as type of enrichment, response to new types of enrichment, and the name of person administering the enrichment. An additional environmental enrichment used in a laboratory setting is a food pellet dispenser that provides positive reinforcement for foraging behavior. Logs should also be maintained by investigators in laboratory settings to document environmental enrichment.

(a) New World Monkeys

- 1) Nest boxes.
- 2) Pseudo-arboreal devices (hanging hoses, PVC pipes).
- 3) Platforms.
- 4) Variation in food:
 - a) fresh fruit (apples, oranges) three times weekly.
 - b) peanut butter in an ice cream cone.
 - c) hand-fed foods such as marshmallows.
- 5) Reversed lighting cycle

(b) Old World Monkeys

- 1) Forage feeding devices charged with raisins, cereal.
- 2) Clutch balls.
- 3) Puzzle feeders
- 4) Television (rotated through the rooms)
- 5) Peripheral suspended activity device
- 6) Variation in food:

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

3) After pairing, animals are monitored for feeding habits, stereotypical behavior, etc., in order to insure that the dominant animal within the pair does not block access to food or water and that the pair remains compatible. Two forage boards per pair are used to ensure equal access to food.

(b) Aotus monkeys are maintained in family groups. Older juveniles are removed after one year and, when possible, pair mated.

(2) Single Housing. All individually housed NHPs have visual contact with each other. In case visual contact cannot be maintained, mirrors will be placed on the wall opposite old world monkeys as a visual enrichment. (New world monkeys do not recognize "self", and therefore, mirrors represent a threat rather than an enhancement of the environment).

(3) Isolation. No animals are isolated from sensory contact with conspecifics unless they are separated due to illness, behavioral problems, or protocol requirements.

(a) If a protocol requires isolation of an animal, the WRAIR Laboratory Animal Care and Use Committee (LACUC) must approve the isolation period and alternative enrichment will be provided to the animal. The animal will be monitored and the exception to policy will be reviewed by the LACUC monthly.

(b) The attending veterinarian has the authority to isolate an animal for medical reasons. If this is necessary, the decision will be reviewed monthly and annotated in the medical records, to include the reason for isolation, anticipated duration of isolation, and plan for enrichment.

c. ENVIRONMENTAL ENHANCEMENT:

(1) Enrichment of the physical environment (primary enclosure) is accomplished utilizing information on species-typical activities and their physiological capabilities. For instance, Aotus monkeys do not have the manual dexterity of an old world monkey. Therefore, "games" requiring dexterity that provide enrichment for rhesus monkeys are inappropriate for Aotus monkeys.

(2) The standards are intentionally broad in order to utilize the imagination of the personnel at each facility. DVM personnel will continue to explore environmental enhancement for each species of monkey housed within WRAIR animal facilities.

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

- a) fresh fruit or vegetables (oranges, apples, bananas, sweet potatoes) three times weekly.
- b) hand-fed peanuts, Prima-Treats
- c) air-popped popcorn prepared in the animal room
- d) yogurt/raisin/peanut butter-filled Kong Toys or cones
- e) Gatorade ice cubes, Gatorade in bottles

d. SPECIAL CONSIDERATIONS:

(1) Animals showing psychological stress through behavior or appearance will:

- (a) be evaluated by a veterinarian.
- (b) be moved within the room or, if necessary, isolated.
- (c) have a high priority for pair housing.

(2) Restraint devices:

(a) Animals will not be maintained in restraint devices unless approved by the WRAIR LACUC. Such restraint will be limited to the shortest period possible.

(b) If restraint is longer than 12 hours, special provisions must be made by the researcher, after consultation with the veterinarian, and with the approval of the LACUC, to provide the NHP the opportunity for unrestrained activity for at least one hour daily. A socialization cage would be ideal to meet this requirement.

2. DOGS AND CATS

a. DEFINITIONS:

(1) Exercise. Physical activity either by free movement in a required cage or removal of the animal from its primary enclosure with section personnel in attendance at all

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

times. Physical activity must be allowed for a minimum of five minutes either in an indoor exercise area or outside on a leash. Personnel monitoring the exercise will provide positive play stimulation during the exercise period. Forced exercise methods or devices such as swimming, treadmills, or carousel-type devices will not meet exercise requirements.

(2) Positive Physical Contact (PPC). Must include petting, stroking, or other touching which is beneficial to the well-being of the animal. This activity must occur for a minimum of five minutes per animal.

(3) Required Space.

(a) The square footage required for an individual dog, using the following formula: measure the length of the dog (tip of nose to base of tail) in inches; add 6 to this figure; multiply this figure by itself (i.e. if the length of the dog is 24 inches, add 6, multiply 30 x 30); divide that figure by 144 (900/144). This is the required square footage for that individual dog.

(b) Currently, required space for cats is 2.5 sq. ft. of floor space per cat. As of February 15, 1994:

1) Each primary enclosure housing cats must be at least 24 in. high (60.96 cm);

2) Cats up to and including 8.8 lbs (4 kg) must be provided with at least 3.0 sq.ft. (0.28 sq. m);

3) Cats over 8.8 lbs (4 kg) must be provided with at least 4.0 sq. ft. (0.37 sq. m).

b. EXERCISE:

(1) Canine runs measure 4 x 10 ft., which provides 40 sq.ft. of space. Based on average size of a beagle and average size of a foxhound, the two breeds historically used in this institute, the canine runs could house five beagles or three foxhounds, each. Depending on space requirements, dogs will be housed either individually or 2-3/ run. This will fulfill the exercise requirement because they are either housed in groups and the runs provide greater than 100 percent of the required space for each dog if maintained separately, or they are housed individually and the space is greater than two times the required floor space for that dog.

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal Environmental Enrichment Program

(2) Dogs assigned to the Department of Instruction are housed as outlined above. They are also walked daily when a class is in session.

(3) Cats are group housed 5-6 per 52.5 cubic foot (10.5 sq. ft. x 5 ft.) cage with an additional 13.5 square feet of resting shelves. A ramp connects the three shelves. This meets the requirements of the current law as well as future requirements effective 15 February, 1994. Cats are provided with toys (balls, chains etc.) in the cages. Twice weekly they are brushed and fed a canned food treat.

c. COMPATIBILITY AND CONFINED HOUSING:

(1) Animals will be monitored for compatibility.

(2) If a protocol requires individual housing of a dog or cat, this can be accomplished.

(3) If a protocol requires confined housing, special provisions must be made by the researcher, after consultation with the veterinarian, and with the approval the LACUC, to provide the dog the opportunity for daily exercise. The frequency, method, and duration of the opportunity for exercise shall be determined by the attending veterinarian in consultation with, and approval by the LACUC.

d. EXEMPTIONS:

(1) The veterinarian may determine that exercise is inappropriate due to health, condition, or well-being. All veterinarian initiated exemptions must be documented in the individual animals medical record. Unless the exemption is permanent, the record must be reviewed monthly, the exemption evaluated, and the decision annotated in the medical record by the attending veterinarian.

(2) LACUC-approved protocols which demonstrate scientific reasons that exercise of the dogs is inappropriate must have a plan for review of this exemption. The LACUC must review its exemption at least annually.

e. POSITIVE PHYSICAL CONTACT:

(1) Canine and feline housing within WRAIR provides physical and sensory contact with other animals. Because sensory contact is provided, positive physical contact with humans is not required. However, DVM personnel will try, given manpower

SGRN-UWN (310-2d)

SUBJECT: WRAIR Policy Letter 93-27, Laboratory Animal
Environmental Enrichment Program

restraints, to provide positive human contact to the dogs and cats
on a daily basis.

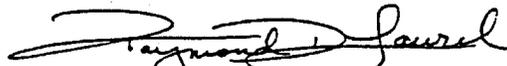
(2) Dogs and cats that are isolated from other animals
will be removed from their primary enclosure, if permitted by the
attending veterinarian, and played with for a minimum of five
minutes, daily.

(3) Dogs assigned to the Department of Instruction
(DOI) are given dog biscuits once per week and groomed as needed.
When a class is in session at DOI, the dogs are given a dog
biscuit three times per week and groomed daily. When a class is
not in session, the dogs are given a biscuit once per week and
groomed as needed.

f. DOCUMENTATION:

A log of all environmental enrichment, positive
physical contact and exercise activities will be posted at the
entrance to each dog and cat housing room. This log will be
available for any personnel involved in these activities to
record the type and duration of activities. A compilation of
these records will be maintained in room #1263 at the leased
facility (Gillette Building) by a senior Animal Care Specialist.

FOR THE DIRECTOR:


RAYMOND D. Laurel
2LT, MS
Adjutant

DISTRIBUTION:
A and B

Appendix N

Animal Test Alternatives Publications

Animal Test Alternatives

REFINEMENT • REDUCTION • REPLACEMENT

edited by

HARRY SALEM, U.S. Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Maryland

November, 1994

376 pages, illustrated

\$135.00

is important reference examines a host of alternatives to the use of animals in research and testing—evaluating the latest developments in the field, indicating future directions, and explaining the regulatory climate that surrounds the techniques presented.

Addresses ocular, dermal, hepatic, renal, respiratory, cardiovascular, and environmental areas of testing and research!

Written by over 75 experts from industry, academia, and government, **Animal Test Alternatives**

covers useful alternative systems, including computer and chemical models, cell and tissue cultures, and the utilization of animals lower on the phylogenetic tree focuses on the traditional three "Rs" of refinement, reduction, and replacement and demonstrates the fourth "R" of responsibility

describes each method in detail and supplies results reviews validation procedures and much more!

With some **670** bibliographic citations and over **100** tables, drawings, and photographs, **Animal Test Alternatives** is an incomparable resource for toxicologists, dermatologists, pharmacologists, cosmetic scientists and technologists, biologists, chemists, biotechnologists, environmentalists, ecologists, government regulators, and upper-level undergraduate and graduate students in these disciplines.

Marcel Dekker, Inc.

270 Madison Avenue, New York, NY 10016
(212) 696-9000

Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
Tel. 061-261-8482

CONTENTS

The Evolution of In Vitro Toxicology in the Pharmaceutical Industry, *Patricia D. Williams*

Relating In Vitro to In Vivo Exposures with Physiologically Based Tissue Dosimetry and Tissue Response Models, *Melvin E. Andersen and Kannan Krishnan*

The Use of Theoretical Descriptors in Quantitative Structure-Activity Relationships, *George R. Famini and Leland Y. Wilson*

Structure-Activity Predictions as Alternatives to Animal Tests, *Kurt Enslein*

The Aquatic Toxicology of Isopropylamine: Comparison of Experimentally Derived Values with Structure-Activity Predictions, *Nancy A. Chester, Mark V. Haley, and Wayne G. Landis*

Structure-Activity Relationships and the Validation of In Vitro Toxicology Tests, *Robert L. Lipnick, Maurice Zeeman, and Joseph A. Cotruvo*

Applications of Liver and Kidney Cell Systems That Can Reduce Animal Usage, *Charles A. Tyson and Carol E. Green*

In Vitro Methods for Hepatotoxic Assessment of Halogenated Fatty Acids, *Nicholas J. DelRaso, Stephen R. Charnel, Merry Jane Walsh, Barry L. Hancock, and William J. Schmidt*

Tissue Slices as an In Vitro Model for Studying Heart, Liver, and Kidney Toxicity, *Paul M. Silber, Tami M. Greenwalt, and Charles E. Ruegg*

Potential for Interspecies Extrapolation of Macrophage Chemiluminescence Data from Immunotoxicology Studies, *Robert S. Anderson, Laurie M. Mora, and Sandra A. Thomson*

An In Vitro System for the Evaluation of Cyanide Ion Binding by Potential Candidate Antidotes, *Lemuel T. Russell, Jurgen D. von Bredow, and James A. Vick*

Munitions Cytotoxicities In Vitro, *W. R. Mitchell, L. M. Dasko-Vincent, and D. R. Wellington*

Neuroblastoma-Glioma Cells as a System for Studying Drug Neurotoxicity, *Arthur D. Weissman, Benjamin R. Crenshaw, Jr., and John E. Johnson, Jr.*

In Vitro Assays for Muscle Irritation, *Sharon J. Northup*

General Overview of In Vitro and Other Alternatives to Skin Toxicity Evaluation, *David W. Hobson*

In Vitro Dermal Toxicity Assays: Validation with Human Data, *Jeff D. Harvell and Howard Maibach*

Animal Test Alternatives

CONTENTS

(continued)

Skin Penetration and Allergic Contact Dermatitis: Historical Perspective, *Francis N. Marzulli*

Toward a Predictive Model for Allergic Contact Dermatitis, *Philip S. Magee, Jurij J. Hostynek, and Howard Maibach*

A Three-Dimensional Human Skin Model for Toxicity Testing, *Dennis Triglia, Tracy Donnelly, Inger Kidd, and Sonia Sherard Braa*

The SOLATEX-PI System: An In Vitro Method to Predict Photoirritation, *Virginia C. Gordon and José Acevedo*

Feasibility of Fluorescence Assays in Human Skin Equivalents with the CytoFluor 2300, *Millard M. Mershon, Charles B. Millard, Jeffrey R. Cook, Laura M. Patrone, Laura S. Rhoads, and Robert G. Van Buskirk*

Ocular Testing: Historical Perspectives, *Van M. Seabaugh*
Overview of In Vitro Ocular Irritation Test Systems and an Evaluation of Their Status, *Shayne C. Gad*

The Role of In Vitro Tests in Assessing the Safety of Cosmetics and Consumer Products, *Thomas J. Stephens and E. Tiffany Spence*

In Vitro and Other Alternatives in Inhalation Toxicology: Monitoring Biological Markers of Cellular and Biochemical Response, *Richard D. Thomas*

Alternatives to In Vivo Toxicological Testing of Rodent Airway Epithelia, *Leah A. Cohn and Kenneth B. Adler*

Understanding Mechanisms of Carcinogenesis Using Rat Tracheal Epithelial Cells In Vitro, *David G. Thomassen*

Development of a Short-Term Bioassay to Assess Pulmonary Toxicity of Inhaled Fibers, *David B. Warheit*

In Vitro Toxicity of Refractory Ceramic Fibers to Chinese Hamster Ovary Cells in Culture, *Georgia A. Hart, Mildred M. Newman, and Thomas W. Hesterberg*

Effects of Phosgene and Perfluoroisobutylene on Permeability of Pulmonary Endothelial Cells in Culture, *Robert J. Werrlein, Janna S. Madren-Whalley, and Stephen Drew Kirby*

In Vitro and Other Alternatives in Cardiovascular Research, *Steven I. Baskin and Harry Salem*

Alternative Tests for Developmental Toxicity, *Thomas J. Flynn*

Frog Embryo Teratogenesis Assay-Xenopus: A Nonmammalian Method for Developmental Toxicity Assessment, *Robert A. Finch, Henry S. Gardner, Jr., and John A. Bantle*

Earthworms as Substitutes for Rodents in Metal Toxicity, *Arthur Furst and Paul K. Chien*

The Development and Validation of the Miniature Swine, Mouse, and Rabbit Models as Alternatives to the Use of the Dog in Drug Testing, *James A. Vick*

Regulatory Requirements for Validation of In Vitro Alternative Tests, *Sidney Green*

Consumer Safety, Harmonization of Test Methods and Classification Systems, and Validation of Alternatives, *Kailash C. Gupta*

Epilogue, *Alan M. Goldberg*

ISBN: 0-8247-9284-X

This book is printed on acid-free paper.



For Credit Card and Purchase Orders, and Customer Service

CALL TOLL-FREE 1-800-228-1160

Mon.-Fri., 8:30 a.m. to 5:45 p.m. (EST)
or FAX your order to 914-796-1772

Of related interest...

In Vitro Toxicity Testing

APPLICATIONS TO SAFETY EVALUATION

edited by

JOHN M. FRAZIER
*The Johns Hopkins University
Baltimore, Maryland*

312 pages, illustrations
\$125.00

"...will prove valuable in bringing in vitro toxicologists up-to-date in research areas other than their own."

—*Alternatives to Laboratory Animal*

CONTENTS

General Perspectives on In Vitro Toxicity Testing, *John M. Frazier*
In Vitro Technology, Trends, and Issues, *Charles A. Tyson and Neill H. Stacey*

Hepatotoxicity, *André Guillouzo*

An Evaluation of In Vitro Models for Assessing Nephrotoxicity, *Patricia D. Williams and Glenn F. Rush*

Neurotoxicity, *Alan L. Harvey*

Cardiovascular Toxicity, *Kenneth Ramos and Daniel Acosta*

Ocular Irritation, *Leon H. Bruner*

Cutaneous Irritancy, *Vincent A. DeLeo*

In Vitro Teratogenicity, *Barbara F. Hales*

Carcinogenicity: The Use of Animal Models and Short-Term Predictive Tests, *David J. Brusick*

Validation of In Vitro Toxicity Tests, *John M. Frazier*

Industrial Applications for In Vitro Toxicity Evaluation: A Tier Test Strategy for Product Safety Assessment, *Shayne Cox Gad*

Regulatory Law and the Use of In Vitro Methods for the Assessment of Various Toxicities, *Sidney Green and June A. Bradlaw*

ISBN: 0-8247-8614-9

Mail today!

ORDER FORM

Mail to: Promotion Dept., MARCEL DEKKER, INC.
270 Madison Avenue, New York, N. Y. 10016

Please send me _____ copy(ies) of *Animal Test Alternatives* edited by Harry Salem at \$135.00 per volume.

Please send me _____ copy(ies) of *In Vitro Toxicity Testing* edited by John M. Frazier at \$125.00 per volume.

Please add \$1.50 for postage and handling per volume; on prepaid orders add only \$.75.

I enclose payment in the amount of \$ _____ by:

check money order Visa

MasterCard (4-digit interbank no. _____) Am.Exp.

Card No. _____ Exp. Date _____

Please bill my company; P.O. No. _____

Signature _____
(must be signed for credit card payment)

Name _____

Address _____

City/State/Zip _____

N. Y. residents must add appropriate sales tax. Canadian customers add 7% GST. Prices are subject to change without notice.

Form No. 119418

Printed in U.S.A.

**EDGEWOOD
RESEARCH,
DEVELOPMENT &
ENGINEERING
CENTER**

ERDEC-SP-012

**PROCEEDINGS OF THE SYMPOSIUM
ON CURRENT CONCEPTS AND
APPROACHES ON ANIMAL TEST ALTERNATIVES**

Harry Salem

RESEARCH AND TECHNOLOGY DIRECTORATE

September 1993

Approved for public release; distribution is unlimited.

**U.S. ARMY
CHEMICAL
AND BIOLOGICAL
DEFENSE AGENCY**



Aberdeen Proving Ground, Maryland 21010-5423

Advances in Animal Alternatives for Safety and Efficacy Testing

EDITORS:

HARRY SALEM, PH.D.
*U.S. Army Edgewood Research
Development and Engineering Center*

SIDNEY A. KATZ, PH.D.
Rutgers University



Taylor & Francis
Publishers since 1798

Appendix O

DoD Meetings on Alternative Methods to Animal Use



EDGEWOOD

RESEARCH, DEVELOPMENT & ENGINEERING CENTER

TECHNICAL PROGRAM

ALTERNATIVES IN THE ASSESSMENT OF TOXICITY: THEORY AND PRACTICE



24-26 May 1994



Edgewood Area Conference Center
Aberdeen Proving Ground, Maryland

Sponsored by:

U.S. Army Edgewood Research,
Development and Engineering Center

In Coordination With:
Association of Government Toxicologists
National Capital Area Chapter - Society
of Toxicology
Sigma Xi
Society of Comparative Ophthalmology



NOTE: CHANGE IN DATE!!!

ANNOUNCEMENT AND CALL FOR PAPERS

4TH BIENNIAL INTERNATIONAL SYMPOSIUM ON

**ALTERNATIVES IN THE
ASSESSMENT OF TOXICITY:
ISSUES, PROGRESS AND OPPORTUNITIES**

12-14 JUNE 1996

**Edgewood Area Conference Center
Aberdeen Proving Ground, Maryland**

Sponsored By:

**U.S. Army Edgewood Research,
Development and Engineering Center**

In Coordination With:

**Department of Defense
Sigma Xi
Society of Comparative Ophthalmology
Others to be Announced**

**PLATFORM AND POSTER PRESENTATIONS WELCOME!
FOR REGISTRATION, PLEASE CALL HEATHER COWAN AT (410) 569-0200**



**EDGEWOOD
RESEARCH, DEVELOPMENT AND ENGINEERING CENTER**

**For Administrative Information Contact:
Heather Cowan or Janice Rhodes, (410) 569-0200**

**For Technical Information Contact:
Dr. Harry Salem, (410) 671-3034**

NO REGISTRATION FEE!!!

Appendix P

Letters from Dr. Martin Stephens



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

February 7, 1992

OFFICERS

K. William Wiseman
Chairman of the Board
Coleman Burke, Esq.
Chairman Emeritus
O.J. Ramsey, Esq.
Vice Chairman
Dr. Amy Freeman Lee
Secretary
John A. Hoyt
President
Paul G. Irwin
Executive Vice President/
Treasurer
Roger A. Kinder, Esq.
Vice President/General Counsel

SENIOR STAFF VICE PRESIDENTS

Patricia Forkan
Senior Vice President
Dale A. Dixon
Government Relations
Patty A. Finch
Youth Education
Dr. Michael W. Fox
Farm Animals & Bioethics
Dr. John W. Grandy
Wildlife & Habitat Protection
Jan A. Harika, Esq.
Environment
Thomas J. Hunt
Controller
Dr. Randall Lockwood
Field Services
Dr. Martin L. Stephens
Laboratory Animals
David K. Wills
Investigations
Phyllis Wright
Companion Animals
Murdaugh Stuart Madden, Esq.
Senior Counsel

DIRECTORS

H. I. (Sonny) Bloch
Coleman Burke, Esq.
Anita Schoonsaker Coupe, Esq.
Irene Evans
Carroll Forgham-Thritt
Regina Bauer Frankenberg
Harold H. Gardner
Alice R. Garay
Dr. Jane Goodall
Leslie R. Inglis
John Kelly
Dr. Amy Freeman Lee
Jack W. Lydman
Virginia Lynch
O.J. Ramsey, Esq.
Marilyn G. Saylor
Robert Sawick
John E. Tall
Terry C. Thomson
Viola Weber
Robert F. Weborn, Esq.
Dr. David Q. Wiebers
Marilyn Wilhelm
K. William Wiseman

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

Dear Harry:

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

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

Again, congratulations.

Best wishes,

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

OFFICERS

K. William Wiseman
Chairman of the Board

Coleman Burke, Esq.
Chairman Emeritus

O. J. Ramsay, Esq.
Vice Chairman

Amy Freeman Lee, Litt.D.
Secretary

John A. Hoyt
Chief Executive

Paul G. Irwin
President and Treasurer

Patricia Forkan
Executive Vice President

Roger A. Kindler, Esq.
Vice President/General Counsel

STAFF VICE PRESIDENTS

Richard M. Clugston, Ph.D.
Higher Education

Patty A. Finch
Youth Education

Michael W. Fox, D.Sc., Ph.D.,
B. Vet. Med., MRCVS
Farm Animals & Bioethics

John W. Grandy, Ph.D.
Wildlife & Habitat Protection

Randall Lockwood, Ph.D.
Field Services

Marc S. Pauthus
Companion Animals

Deborah J. Salem
Publications

Martin L. Stephens, Ph.D.
Laboratory Animals

David K. Wills
Investigations

Murdaugh Stuart Madden, Esq.
Senior Counsel

DIRECTORS

H. I. (Sonny) Bloch

Donald W. Cashen

Anita Schoemaker Coupe, Esq.

Judi Friedman

Harold H. Gardiner

Alice R. Garey

Jane Goodall, Ph.D.

Leslie R. Inglis

Jennifer Learning, M.D.

Amy Freeman Lee, Litt.D.

Eugene W. Lorenz

Jack W. Lydman

Virginia S. Lynch

Thomas L. Meinhardt

O. J. Ramsay, Esq.

James D. Ross

Marilyn G. Seyler

John E. Taft

Terry C. Thomason

Carroll Thrift

Robert F. Welborn, Esq.

David O. Wiebers, M.D.

Marilyn E. Wilhelm

K. William Wiseman



June 1, 1994

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

Dear Harry:

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

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

Let me know what you think.

Best wishes.

Sincerely,

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

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

Appendix Q

**National Research Council Fellowship in Alternatives
Research at the U.S. Army Edgewood Research, Development,
and Engineering Center**

Resident Research Associateships

Postdoctoral and
Senior Research Awards

1995

OPPORTUNITIES FOR RESEARCH

at the

U.S. ARMY EDGEWOOD RESEARCH, DEVELOPMENT, AND
ENGINEERING CENTER
Aberdeen Proving Ground, Maryland

administered by the
NATIONAL RESEARCH COUNCIL
Washington, DC

Life Sciences Research and Testing: Alternatives to Animal Testing - Physiologically Based Pharmacokinetic Modeling, Dose Response Extrapolation

H. Salem

11.01.04.10

Research opportunities are available in the following areas: (1) toxicology, (2) pharmacology, (3) toxinology, (4) alternatives to animal testing, (5) physiologically based pharmacokinetic modeling extrapolating animal data to human effects, and (6) dose-response extrapolation. Studies are conducted to determine the efficacy and safety of chemicals of military interest using classical toxicology/pharmacology by all routes of administration, with special emphasis on inhalation. Fate and effects studies, as well as aquatic and environmental toxicology are also conducted.

The Life Sciences Department is comprised of the following teams: Biosciences, Environmental Technology, Inhalation Toxicology, Respiratory Protection, and Veterinary Services. Our laboratories are accredited by the American Association for Accreditation of Laboratory Animal Care. In addition, studies are examined by a quality assurance unit to ensure that they comply with good laboratory practices.

Resident Research Associateships

Postdoctoral and
Senior Research Awards

1996

OPPORTUNITIES FOR RESEARCH

tenable at the

U.S. ARMY EDGEWOOD RESEARCH, DEVELOPMENT, AND
ENGINEERING CENTER
Aberdeen Proving Ground, Maryland

administered by the
NATIONAL RESEARCH COUNCIL
Washington, DC

Life Sciences Research and Testing: Alternatives to Animal Testing - Physiologically Based Pharmacokinetic Modeling, Dose Response Extrapolation

H. Salem

11.01.04.10

Research opportunities are available in the following areas: (1) toxicology, (2) pharmacology, (3) toxinology, (4) alternatives to animal testing, (5) physiologically based pharmacokinetic modeling extrapolating animal data to human effects, and (6) dose-response extrapolation. Studies are conducted to determine the efficacy and safety of chemicals of military interest using classical toxicology/pharmacology by all routes of administration, with special emphasis on inhalation. Fate and effects studies, as well as aquatic and environmental toxicology are also conducted.

The Life Sciences Department is comprised of the following teams: Biosciences, Environmental Technology, Applied and Inhalation Toxicology, and Biotechnology and Aerosol Sciences. Our laboratories are accredited by the American Association for Accreditation of Laboratory Animal Care. In addition, studies are examined by a quality assurance unit to ensure that they comply with good laboratory practices.