
**DEPARTMENT OF DEFENSE
ANIMAL CARE AND USE PROGRAMS
FISCAL YEAR 2002–2003**

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LIST OF ACRONYMS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
AALAS	American Association for Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
AFIP	Armed Forces Institute of Pathology
AFRRI	Armed Forces Radiobiology Research Institute
APHIS	Animal and Plant Health Inspection Service
AWA	Animal Welfare Act
CDMRP	Congressionally Directed Medical Research Programs
DARPA	Defense Advanced Research Projects Agency
DoD	Department of Defense
FY	Fiscal Year
GEIS	Global Emerging Infections Surveillance and Response System
GME	Graduate Medical Education
HIV	Human Immunodeficiency Virus
IACUC	Institutional Animal Care and Use Committee
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILAR	Institute of Laboratory Animal Research
LAM	Laboratory Animal Medicine
NHP	Nonhuman Primate
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
PL	Public Law
RDT&E	Research, Development, Test, and Evaluation
USDA	U.S. Department of Agriculture
USUHS	Uniformed Services University of the Health Sciences
VEE	Venezuelan Equine Encephalitis
WRAIR	Walter Reed Army Institute of Research

SECTION 1

INTRODUCTION

The fiscal year 2002–2003 (FY02–FY03) report on Department of Defense (DoD) Animal Care and Use Programs was conducted by the Director, BioSystems, Office of Director, Defense Research and Engineering. In addition to a general overview, this report provides a summary of DoD animal use with respect to research and medical training activities. It also addresses the underlying rationale, or benefits, of this animal use and efforts by the DoD to implement animal use alternatives.

1.1 DOD POLICY GOVERNING ANIMAL RESEARCH

The DoD is committed to full ethical and regulatory compliance for its animal-based research programs. It has been proactive in improving the fixed infrastructure and span of control necessary to ensure compliant, responsible, and efficient execution of programs and maximize oversight of diverse and varied missions. The Department has aggressively implemented focused programs and policy documents that optimize the standardization of animal care. This enhanced standardization and oversight have improved a historically good system and made it an outstanding model to be emulated.

In 1995, the DoD revised and implemented the directive dealing specifically with animal care and use ([DoD Directive 3216.1, “Use of Laboratory Animals in DoD Programs,” 1995](#)). This directive strengthens and clarifies requirements for nonaffiliated membership in institutional oversight and directs all DoD animal use facilities that maintain animals for research, development, test, and evaluation (RDT&E) and training to apply for Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accreditation. DoD veterinarians, researchers, and policymakers continue in their efforts to be proactive in maintaining the highest level of accountability for animal use.

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.](#)” This 1995 Policy Memorandum specifies training requirements for nonaffiliated DoD Institutional Animal Care and Use Committee (IACUC) members and implements a [standard format for animal use protocols](#), a [standard checklist for IACUC inspections](#), and a standard reporting requirement for all animal use research to support the [Biological Research Database](#), which is publicly accessible. All animal research must conform to requirements of the 1966 Animal Welfare Act (AWA) (Public Law [PL] 89-544) as amended in 1970 (PL 91-579), 1976 (PL 94-279), 1985 (PL 99-198), and 1990 (PL 101-624) as well as the National Research Council’s Guide for the Care and Use of Laboratory Animals, (7th rev. edition, 1996), the [U.S. Government Principles for Animal Use \(1985\)](#), and the requirements of the applicable regulations of the U.S. Department of Agriculture (USDA).

Mice and rats are the most commonly used species in DoD research. Although the AWA and its implementing regulations currently exempt these species, the DoD has long afforded them, along with all other vertebrates, including fish and frogs, the same consideration given to nonexempt species under the AWA. In implementing a full accounting of the use of mice and rats, the DoD is relatively unique in the scientific research community. At the same time, DoD researchers have aggressively developed novel procedures to replace, reduce, and refine the use of animals in their research.

1.2 REQUIREMENTS NECESSITATING THE USE OF ANIMALS BY THE DOD

The DoD’s use of animals in RDT&E and in medical education and training programs is critical to the sustained technological superiority in military operations in defense of our national interests. The DoD programs that are dependent on animal use ultimately translate into improved military readiness as well as a reduction in morbidity and mortality associated with military operations. Many of these programs directly contribute to Force Health Protection, allowing our forces to operate in and survive the numerous and various hazards they face around the world. DoD researchers are committed to accomplishing this goal, and it is important to emphasize that, as in nonmilitary research programs, the involvement of animals in research cannot always be avoided.

DoD research has benefited greatly from animal use alternatives such as nonliving systems, cell and tissue culture, and computer technology. However, complex human organ system 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 and medical personnel, situations remain for which there are no acceptable nonanimal alternatives. The DoD continues to embrace new advances, technologies, and breakthroughs in animal use alternatives such as the widespread replacement of animals by highly sophisticated computer software in surgical training. Section 3 of this report provides a summary of the many animal use alternatives being explored and implemented in DoD institutions.

Disease remains a major cause of disability and sometimes death in military operations and conflicts. Today, overseas humanitarian and peacekeeping operations expose our troops to endemic pathogens to which their immune systems are naive. Soldier health and performance can be compromised by a variety of diseases for which there are no effective preventive or therapeutic countermeasures. Research toward the development of effective pretreatments, vaccines, and therapies requires the use of specific animal models in assessing safety and efficacy.

Operations Desert Storm and Desert Shield in the Persian Gulf, Restore Hope in Somalia, and the current operations in Afghanistan and Iraq have yielded outbreaks of respiratory and diarrheal diseases such as shigellosis, and parasitic diseases such as leishmaniasis threatened the health and well-being of our troops. The DoD also invested considerable effort to address concerns over the long-term effects of various environmental, physical, and medical factors associated with the Persian Gulf conflict. Even as political and military conflicts conclude, issues concerning the health and well-being of military personnel extend far beyond the immediate scope of the battlefield.

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 therapeutics are needed for protection against numerous militarily relevant diseases and threats, many of which can result in potentially fatal diseases or conditions that have no known treatments, therapies, or cures. Ethical responsibilities, as well as regulatory requirements of the U.S. Food and Drug Administration, necessitate that candidate vaccines, drugs, and devices be demonstrated safe and efficacious in laboratory animal models prior to initiation of human use protocols whenever possible. Drug efficacy screens are generally conducted at the lowest possible phylogenetic level (i.e., in rodents). Given that drug response is often highly species specific, promising drugs are subsequently tested in nonhuman primates (NHPs) before commencing the final stages of vaccine and drug development, wherein large-scale safety and efficacy testing is usually conducted using human volunteers.

The DoD must develop the materiel and technological means to provide critical and immediate battlefield injury care to service men and women. This is often provided by field medical personnel in an austere, harsh, and hostile environment, hours away from full hospital medical care. This contrasts markedly with medical facility counterparts in the civilian community that generally possess well-appointed emergency medicine and trauma management systems. A domestic, low-velocity projectile gunshot patient in a modern civilian shock and trauma center will be supported and managed by a full complement of medical and surgical staff, and a full complement of pharmaceutical supplies. The combat casualty may be supported by only a single field medic or fellow Soldier and the medical supplies, experience, and expertise this person has. No *in vitro* model can simulate the range of effects of multiple organ failure or shock that so often follows physical trauma.

There are numerous research areas, including medical chemical and biological warfare defense, where animal-based studies are particularly critical because the conduct of human use protocols is simply not possible in the search for understanding and developing protection against many highly lethal agents. Ethical considerations severely restrict or preclude the use of human studies in this research area. The world is no longer a place where the deadly chemical poisons and pathogens of mass destruction are controlled by the infrastructure of national governments. Terrorist organizations have demonstrated a ruthless disregard for human life, fomenting mass murder on a previously unimaginable scale. Rogue nations, some with weapons of mass destruction, are in a position to transfer these destructive technologies to organizations seeking to attack U.S. civilians and military personnel. Terrorists have already shown their ability to develop large-scale, clandestine chemical and biological agent manufacturing facilities in Japan. Both chemical and biological weapons were released in that nation, and U.S. civilians have been targeted with anthrax. The sheer magnitude of these threats underscores the need to develop protective medical countermeasures for both military and civilian personnel. The DoD is charged with

the responsibility of identifying and developing these defensive countermeasures to protect the nation, and carefully regulated animal studies are critical to the success of biomedical research programs supporting, for example, the development of safe and effective vaccines for anthrax.

The responsibility of the DoD to maintain the health of men and women and their families where they work, whether on military installations, the battlefield, or in peacekeeping missions around the world, underlies the need for the DoD to conduct research and to train and educate military health care providers. Clinical investigation programs at medical treatment facilities support postdoctoral graduate medical education (GME) programs, in which physicians receive residency training in special areas such as orthopedics, surgery, and emergency critical care. To be certified, the GME programs must demonstrate that a medical facility has programs to provide research opportunities for both staff and students. These clinical investigation programs provide training in the performance of research involving both laboratory animals and human subjects. This combined capability increases the opportunities for staff and GME students and significantly enhances their training, thus enabling the warfighter to receive the best care possible. This capability also increases opportunities for patients who desire to participate in research protocols such as the Multicenter Oncology and Pediatric Oncology protocols. In this regard, Congress has mandated that the DoD work closely with the National Institutes of Health (NIH) to provide more opportunities for DoD beneficiaries to participate in NIH-sponsored protocols. Many of the clinical investigation training protocols, such as surgical skills training for microvascular or reproductive surgery, support GME programs that follow requirements set by the American College of Surgeons. These courses provide essential opportunities for the training of medical personnel who will work in both military and civilian sectors. Programs using animals for GME training are also subjected to veterinarian oversight, and these animals are maintained in facilities accredited by the AAALAC International.

The use of animals is also important in the DoD's nonmedical programs. These studies include the development of biological sensors, sonar, echolocation, biorobotics, aviation construction materials, and hearing and eye protection systems. There are also nonmedical studies to understand learning and memory physiology in an attempt to model the brain's circuitry for advanced data processing computers and robotic machinery. These advanced computers and robots will eventually reduce the risks that our service men and women encounter in their daily duties. In performing marine biology research to better understand military working marine mammals, the DoD funds unique research that increases our understanding of these fauna. Marine mammals are investigated to determine their auditory detection thresholds in marine use as sentries. Studies of biosonar systems are conducted to enhance the use of military marine mammal systems for mine detection and retrieval, personnel detection, and reconnaissance.

1.3 BENEFITS OF ANIMAL RESEARCH

DoD personnel and DoD-funded contractors provide the new or improved capabilities needed to address medical and nonmedical challenges of the future through the efforts of internationally renowned medical and scientific experts working in state-of-the-art facilities and in the field. The Department conducts and resources RDT&E and training missions to sustain the operational capabilities of today's service men and women. Many of these programs require the use of animals to meet mission requirements and result in benefits for both the military and civilian sectors (Tables 1-1, 1-2, and 1-3). The military benefits from supporting research programs in areas that currently threaten military personnel, such as combat trauma, chemical and biological agents, infectious diseases not endemic to the United States, directed energy, and occupationally unique health hazards from military operations and environmental extremes. These research programs contribute significantly to the readiness and sustainment of the DoD's warfighting capability and focus heavily on the prevention of casualties. These benefits reflect the diversity of DoD research efforts in support of joint warfighter needs ([Benefits of DoD Intramural RDT&E and Training](#)).

It is important to recognize that DoD research requirements benefit civilians both in the United States and in the world community. The DoD indirectly or directly advances understanding of our knowledge of cardiovascular disease, trauma care and treatment, respiratory injuries, burns, and specific surgical procedures. The DoD's role in these areas is critical because some traditionally receive only modest funding support in civilian research programs. Marine researchers and policymakers also benefit from DoD marine mammal research through its indirect contribution of a better understanding of the impact on marine mammals of noise pollution from ships.

With the end of the Cold War, Congress has added to the DoD's research portfolio the task to manage medical research that directly benefits the civilian population, such as research in breast, prostate, and ovarian cancers. These research programs, developed with guidance from the National Academy of Sciences, account for a considerable portion of DoD extramural animal research, and are having an immense and positive impact on the understanding, prevention, and treatment of these cancers and other diseases. Transgenic mice, for example, are critical for determining highly specific gene effects on the development and progression of cancers. No in vitro system exists that can model the extremely complex cellular and molecular "crosstalk" between tissues and cells, and cell cultures are highly prone to artifactual observations stemming from the genetic changes required to establish a permanent cell line and by cells growing and developing in a completely unnatural extracellular context.

The infectious disease and medical chemical and biological defense research programs are primarily designed to develop countermeasures to potential threats to U.S. military personnel who must operate in a global setting. In FY02–FY03, these research programs were awarded patents as shown in Table 1-2. While the underlying requirement for disease research is to protect U.S. military men and women, it should be noted that there is an indirect benefit of the DoD's research to the broader world community. The scant resources of many poorer nations are directed at basic survival needs such as food and medicine, and not research. Because U.S. troops must operate in a worldwide theater, the DoD has had a long-standing commitment to the development of countermeasures against malaria, the disease that annually kills more people than any other. DoD scientists also collaborate closely with the NIH in important areas of study, including the development of vaccines and treatments for malaria and human immunodeficiency virus (HIV) infection. In addition, there are many examples of direct humanitarian benefits of the DoD investment and collaborative efforts with other nations to improve the quality of life of both humans and animals. Several prime examples of the humanitarian benefits of DoD research efforts are noted in Table 1-3.

Another benefit of animal research is the development of medical products that can be implemented in the battlefield to save lives. These currently range from remote sensors to monitor Soldier health, the development of blood substitutes and agents for hemorrhage control, to the prevention of shock. Clinical trials under way are addressing the efficacy of vaccines and/or treatments for malaria, diarrhea, HIV, encephalitis, adenoviral infection, and hepatitis E.

Besides the medical benefits of animal research, there are many nonmedical and training benefits. The development of biosensors and the identification of environmental hazards benefit military and civilian communities alike. The DoD has many exceptional medical and scientific educational programs that train both medical personnel and scientists. While these people are in the military, the DoD reaps the benefit of this training; once they leave the military and apply their training in the private sector, the civilian community realizes this benefit. The DoD's development of alternatives to reduce or replace animals provides an extra value to both communities and to animals. Also, refinement research results in more humane methods of performing research that is applied in many types of research settings.

The benefits of scientific research are customarily shared in publications. In FY02–FY03, the DoD reported nearly 700 publications in scientific journals, proceedings, technical reports, books, and book sections from RDT&E efforts that required the use of animals.

Table 1-1 Animal Use Benefits**Medical RDT&E**

- Development and evaluation of malaria, HIV, and anthrax vaccines
- Development of an intranasal meningococcal vaccine
- Patenting of a portable hand pump to evacuate air and blood from body cavities
- Evaluation of the acute effects of laser exposure
- Research on the mechanisms of occupational and chronic fatigue
- Quantification of munitions compound toxicity on wildlife
- Research on the pathogenesis and treatment of hearing loss
- Identification of markers to determine the magnitude of blunt trauma
- Development of new and effective drugs against chemical warfare agent exposure
- Determination of the molecular mechanisms, detection, and treatment of breast, prostate, and ovarian cancers, and neurofibromatosis
- Researching methods of wound debridement on the battlefield
- Identification of the genetic basis for the 24-hour, or circadian, body clock
- Optimization of sleep management relative to performance

Clinical Investigations

- Clinical research on the development and testing of HIV vaccines
- Development of drugs to enhance wound healing
- Development of treatments for exposure to excessive noise
- Treatment and prevention of hemorrhagic shock
- Treatment and repair of duodenal (upper intestinal) injuries
- Development of an animal model for colon cancer
- Treatment and prevention strategies for post-traumatic stress disorder
- Development of therapeutic treatment of hypothermia in the field
- Development of an improved system for the treatment of bone infections (osteomyelitis)

Training/Instructional

- GME training
- Training of surgical residents in a variety of critical skills
- Advanced trauma life support and medical emergency training
- Veterinary personnel medical emergency training
- Training for research and animal care personnel to improve handling techniques and protocol procedure performance

Nonmedical RDT&E

- Updating of the national and international laser safety standards
- Identification of environmental and human health risk factors
- Developing methods and technologies for toxicity testing
- Developing preventive measures for environmental toxins
- Developing biomonitoring systems
- Evaluating toxicological hazards of occupational chemical exposure

Table 1-2 Patents Resulting from Animal Use Research in FY02–FY03

- Three candidate vaccines to protect against dengue virus
- A microsphere delivery system for the controlled release of anti-inflammatory drugs
- Several antimalarial drugs
- A vaccine against gram-negative bacteria
- A portable hand pump to evacuate air and blood from body cavities
- A detoxifying sponge that inactivates chemical warfare agents
- A compound that helps protect against the effects of head injury
- Drugs for the inhibition and treatment of tetanus and botulinum toxins

Table 1-3 Humanitarian Benefits of DoD Research Efforts

In Peru, the DoD has investigated the epidemiology of viral hemorrhagic and encephalitic diseases among civilians and deployed military troops in Peru. This research has demonstrated that the arthropod-borne viruses most commonly associated with human disease in the Amazon region were dengue, Oropouche, and Venezuelan equine encephalitis (VEE). In addition, Yellow Fever, Mayaro, VEE, and one case by an apparently new Phlebovirus (family Bunyaviridae) were isolated from febrile patients in an outbreak in the high jungle near Cusco, Peru. This was the first isolation of Maguari virus, which is associated with human disease.

The DoD performs critical diagnostic analyses of suspected disease outbreaks in the United States and overseas and provides vaccine materials for both humans and animals in emergency settings. DoD research facilities were at the forefront of efforts to diagnose and control outbreaks of: (1) deadly hantavirus infection among Navajo Native Americans in 1993; (2) Rift Valley fever in Egypt in 1993; (3) VEE in people and horses in central and South America in 1995; (4) Ebola and related viruses in Zaire in 1995; (5) West Nile virus in New York citizens, horses, and birds in 1999; and (5) anthrax distributed by mail in Washington, DC in 2001. Over the years, the DoD has developed effective vaccines for numerous infectious agents that are variously associated with Rift Valley Fever, VEE, Ebola virus, hemorrhagic fever, plague, dengue, anthrax, botulism, tick-borne encephalitis and hepatitis A, and staphylococcus enterotoxins.

Malaria is one of the world's greatest killers, and the DoD's fielding of new drugs is critical in the face of the development of resistance to currently fielded drugs. With some notable exceptions, civilian drug developers have shown reluctance to invest in malarial vaccines because of a low likelihood of fiscal return. The Army has partnered with GlaxoSmithKline, Inc. in developing a bivalent vaccine, designed to protect against both malaria and hepatitis B, which may be more commercially viable. Army antimalarial researchers have tested more than 500,000 drugs and other substances for activity against malarial pathogens.

The DoD collaborated with the Argentine government in the development of the Junin vaccine that has provided critical, 98% effective protection for more than 120,000 individuals in endemic areas of Argentina against the ravages of Argentinean hemorrhagic fever.

1.4 SCOPE OF REPORT

This report covers animal research in the context of education, training, and RDT&E both conducted and sponsored by the Department for FY02–FY03. The two major components of the FY02–FY03 report are: (1) a summary of animal use with regard to species, DoD Components, research areas, and USDA pain categories (Section 2) and (2) DoD initiatives to promote alternative methods that replace, reduce, or refine animal use (Section 3). 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. Information was solicited and received from DoD military commands, agencies, and activities and from non-DoD organizations involved in DoD-supported animal care and use programs. For the purpose of this report, an intramural program represents research performed at a DoD facility funded by either DoD or non-DoD funds while an extramural program represents research performed by a contractor or grantee that is funded by the DoD. In FY02–FY03, data were acquired from 33 DoD organizations and about 1,500 extramural activities.

Additional information regarding the DoD Animal Care and Use Program can be found at <http://www.dtic.mil/biosys>. Policies, the standard research protocol format, the Biomedical Research Database (containing descriptive summary information of current DoD animal research projects), and prior reports are provided at this website.

1.5 CONCLUSION

It remains essential to use animals in DoD RDT&E, education, and training to protect the health and lives of military personnel. Although alternatives to animal use will continue to be vigorously sought and applied as possible, the complex interactions of organ, tissue, cell, disease agents or processes, and environment make the continued judicious use of animals in DoD programs necessary. Animals are used in research only when scientifically acceptable alternatives are not available. The DoD is committed to full ethical responsibility and regulatory compliance for its animal-based research programs. The Department's animal care and use requirements are as strict or stricter than those required of non-DoD, government-funded, or public and private research institutions. DoD policy directs all facilities maintaining animals for use in research and training to apply for AAALAC accreditation, and the DoD has established effective programs to replace, reduce, and refine its current use of animals.

SECTION 2

DoD ANIMAL USE PROFILES

The information presented in this section provides profiles on the reported use of animals with regard to DoD Components, species, and animal use and USDA pain categories.

2.1 METHODS

Information was solicited and received from DoD Components and DoD-funded organizations involved in animal care and use programs located both in and outside of the United States for FY02–FY03. These 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 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.

2.1.1 Animal Use Profiles

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

For the purposes of the DoD animal care and use reporting requirement, an animal was defined as any live, nonhuman vertebrate used for RDT&E. Only live animals that were either on hand in the facility or acquired and used during FY02–FY03 are included. Carcasses, animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This report 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. Breeding animals or animals on hand during FY02–FY03 but not actually used during the fiscal year are not included in the numbers reported here.

2.1.2 Animal Use Categories

The DoD uses a system for classifying all animal use that is broken down into 7 categories and 20 subcategories (see Table 2-1). Five primary categories (medical, clinical investigations, adjuncts/alternatives, training/instructional, and nonmedical) are represented among FY02 and FY03 activities. In past years, if one of the five primary [Animal Use Categories](#) did not adequately describe the animal use within a particular work effort, the animal was placed in the Other Animal Use category (Category O). However, for this FY02–FY03 report, the relatively few Category O projects were found to reasonably fit under the better-defined animal use Category N4 (Other Nonmedical RDT&E, which includes research in the neurosciences) and Category A (Adjuncts/Alternatives to Animal Studies). It should be noted that no animals in any of these areas were reported as used for the development or testing of offensive weapons (Category N, subcategory N3).

Table 2-1 Animal Use Categories and Subcategories

<p>MEDICAL (M) RDT&E 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</p> <p>CLINICAL INVESTIGATIONS (C) C1: Clinical Medicine C2: Clinical Surgery C3: Other Clinical Investigations</p> <p>ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A) A1: Adjuncts to Animal Use Research A2: Alternatives to Animal Research A3: Other Alternatives/Adjuncts</p>	<p>TRAINING/INSTRUCTIONAL (T) T1: Training, Education, and/or Instruction of Personnel T2: Other Training/Instruction</p> <p>NONMEDICAL (N) RDT&E N1: Physical Protection N2: Physical Detection N3: Offensive Weapons Testing N4: Other Nonmedical RDT&E</p> <p>CLASSIFIED SECRET OR ABOVE STUDIES (S) Studies classified secret or above</p> <p>OTHER ANIMAL USE (O) Other animal use purposes</p>
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2.1.3 USDA Pain Categories

The USDA requires that all institutions using regulated animals for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. In this report, animals are assigned to one of three USDA pain/distress categories (Table 2-2). As noted above, this report includes animal species not regulated by the AWA and its implementing regulations.

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

<p>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.</p> <p>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.</p> <p>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.</p>

The animals reported in Column C of the USDA report are those used in a procedure that would reasonably be expected to cause not more than slight or momentary pain and/or distress in a human being to whom that procedure was applied. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood-sampling techniques (excluding intracardiac blood sampling), injections, and tattooing.

The animals reported in Column D of the USDA report are those for which pain is alleviated or controlled by appropriate anesthetic, analgesic, or tranquilizing drugs. 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, or might experience, more than slight or momentary pain or distress because the administration of pain-relieving drugs would adversely affect the study. 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. Included under Column E are toxicity studies using thousands of fish that, while showing no signs of distress, must be assigned to this category for lack of an effective way to monitor discomfort.

All procedures that involve animals reported under USDA Pain Category Column D or E are extensively reviewed during the protocol approval process. Prior to formal protocol review, a veterinarian with experience and/or training in laboratory animal medicine (LAM) must review all procedures. In addition, the primary investigator must write a justification for all procedures for animals reported under Column E. The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the IACUC must review and approve all procedures before the study begins.

2.2 RESULTS/DISCUSSION

2.2.1 General Results

Since 1999, the number of animals reported per year has been relatively steady, averaging at about 342,000 (Figure 2-1). Total annual use can show significant change over the years with the transient implementation of specific extramural research projects that employ large numbers of animals or with the conduct of intramural or extramural testing programs. The total values for FY02 and FY03 are 355,150 and 335,233, respectively. It should be noted that these numbers include rats, mice, birds, frogs, and fish. None of these animals are required to be reported under the AWA. Using the limited definition of *animal* under the AWA, the DoD would report much lower totals of 24,287 and 25,396, respectively, comprising only 6.9% and 7.6% of the true total of animals actually used in FY02 and FY03. Hence, the DoD's nonrestrictive definition of *animal*, which includes all vertebrates from fish to NHPs, reflects a much higher level of accountability.

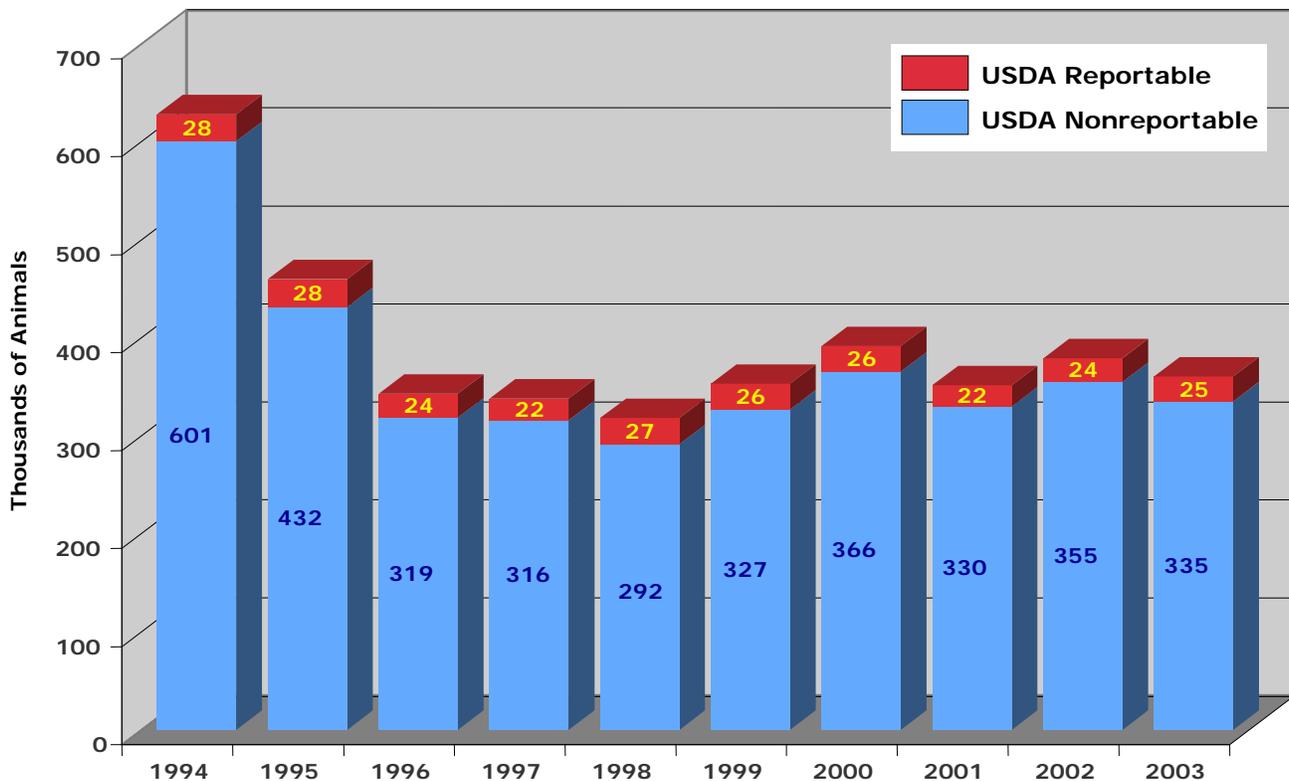


Figure 2-1 Animal Use by Fiscal Year

In FY02 and FY03, 158,130 and 124,945 animals, respectively, were used in intramural research programs, and 197,020 and 210,288, respectively, were used in extramural grants or contracts (Figure 2-2). The FY03 intramural total of 124,945 reflects a steady reduction in intramural animal use since the 187,200 level seen in both 1999 and 2000. This contrasts with a nearly 32,000 increase in extramural animal use since 2000. Both intramural and extramural numbers remain considerably lower than the 1994 peak usage values of 268,091 and 332,592, respectively. In FY03, intramural and extramural activities reflect respective declines of 54% and 37% relative to FY94.

Given that the level of funding for extramural programs varies from year to year depending on congressional funding and DoD priorities, the total number of extramural projects employing animals fluctuates with changes in the number of contracts and grants awarded. Furthermore, many extramural research projects use animals only during the final years of a project after the preliminary demonstration of a theory or concept in vitro.

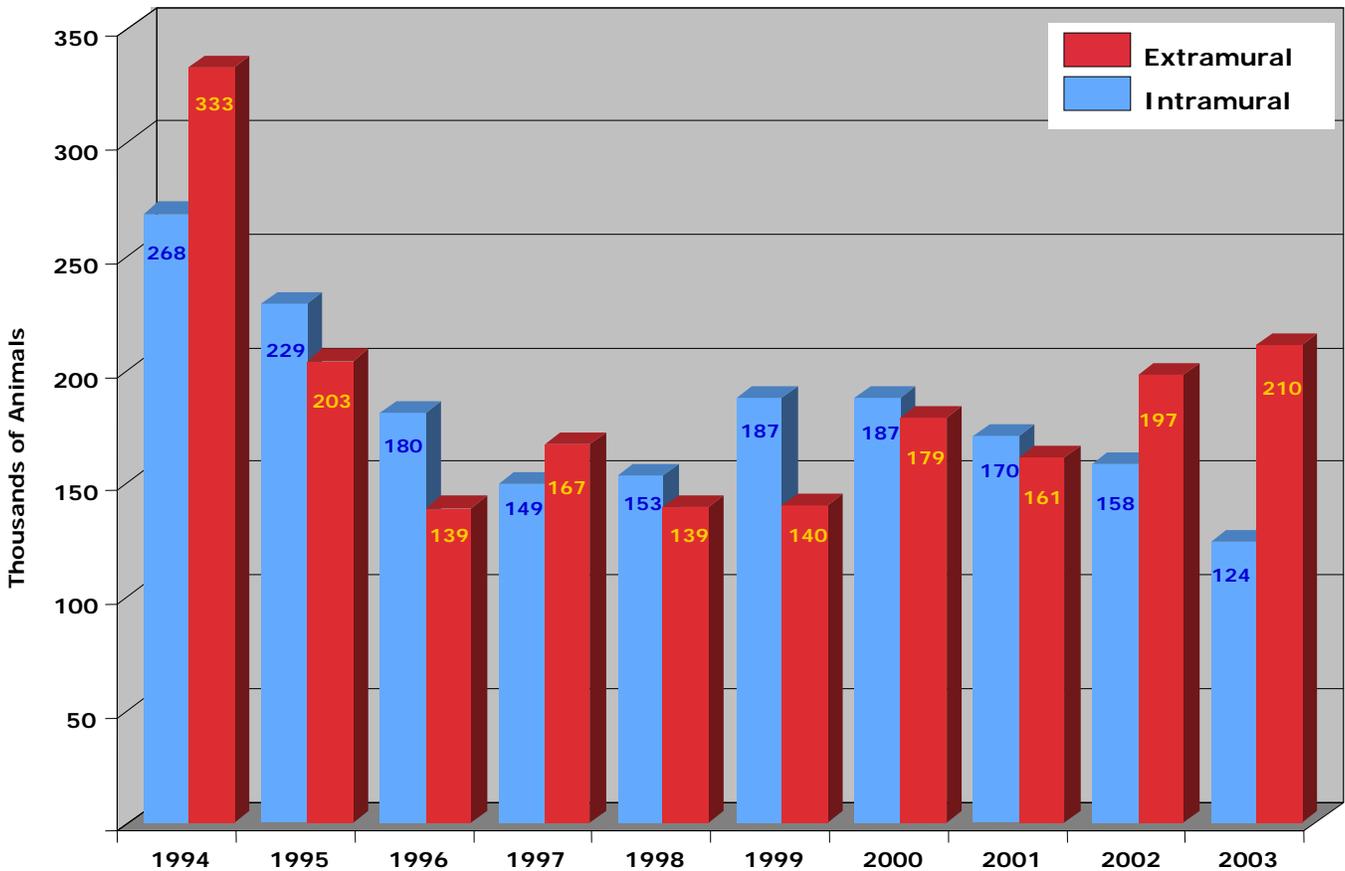


Figure 2-2 Intramural/Extramural Animal Use by Fiscal Year

Since FY94, there has been a remarkable decrease in animal use in intramural and extramural activities that directly support DoD mission requirements. Beginning in FY95, Congress directed the DoD to implement the Congressionally Directed Medical Research Programs (CDMRP) with an initial infusion of more than \$225 million. CDMRP funds non-DoD mission-required research that comprise nearly all of DoD animal use Category M8 (Other Medical RDT&E). The vast size of this congressionally directed extramural program has masked the decline in extramural DoD mission-directed animal use. Overseen by the Army, congressionally mandated biomedical research efforts have received steady funding ever since, accounting for nearly 50% of all extramural research in FY03. CDMRP used more than 101,000 and 90,000 animals in FY02 and FY03, respectively, corresponding to 28% and 27% of annual DoD totals. The FY03 numbers reflect a 3-year decline in intramural animal use, primarily a simple reflection of turnover in mission-related activities. When considering only DoD mission-required research, there is a large decline in extramural animal use since FY94 exceeding 200,000 animals.

2.2.2 Animal Use by Military Department

Information concerning total reported DoD use of animals by each of the three Military Services and the remainder of the DoD is presented in Figure 2-3. The category, “Other DoD,” includes the Uniformed Services University of the Health Sciences (USUHS), Defense Advanced Research Projects Agency (DARPA), Armed Forces Radiobiology Research Institute (AFRRI), and Armed Forces Institute of Pathology (AFIP). Figures 2-4 and 2-5 show the intramural and extramural animal use by the Military Services and “Other DoD,” respectively.

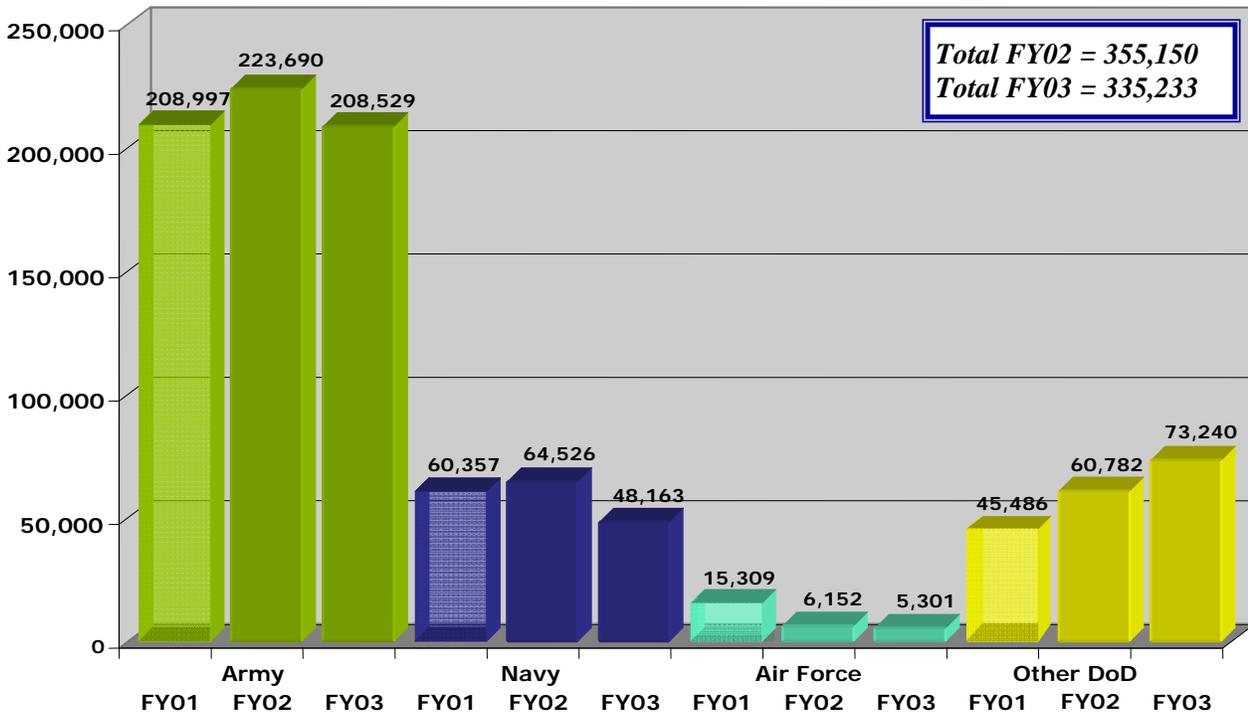


Figure 2-3 Intramural and Extramural Animal Use by DoD Components for FY02–FY03 (FY01 for comparison)

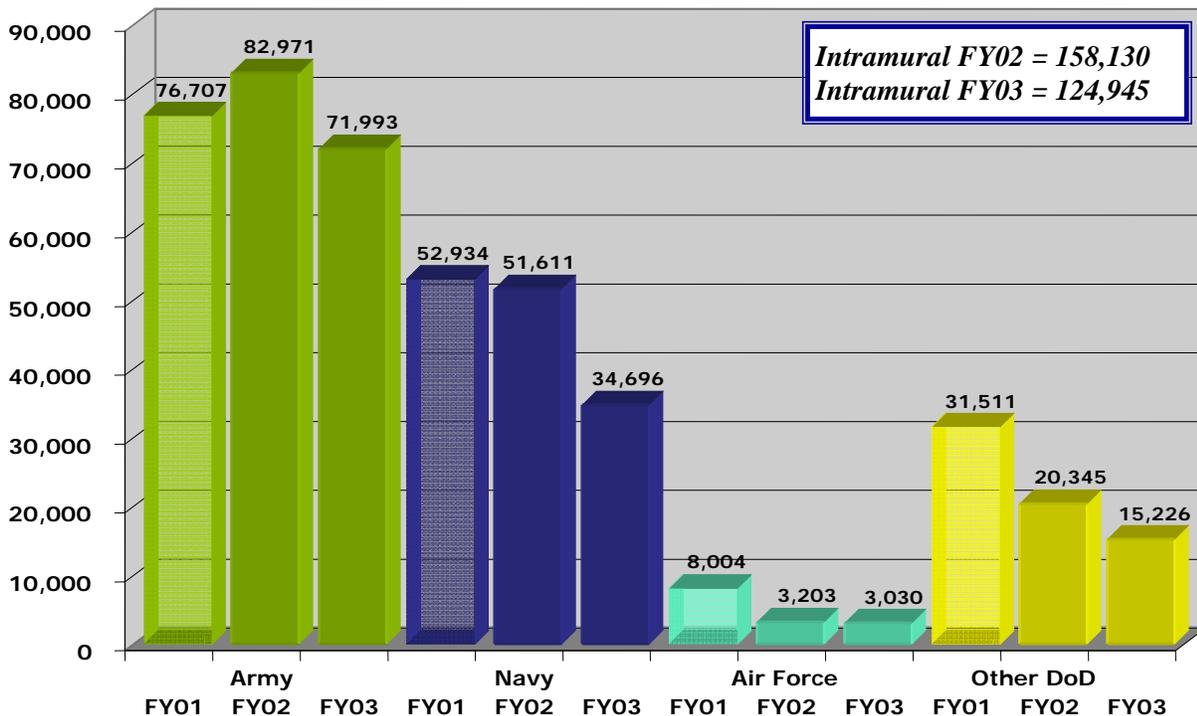


Figure 2-4 Intramural Animal Use by DoD Components for FY02–FY03 (FY01 for comparison)

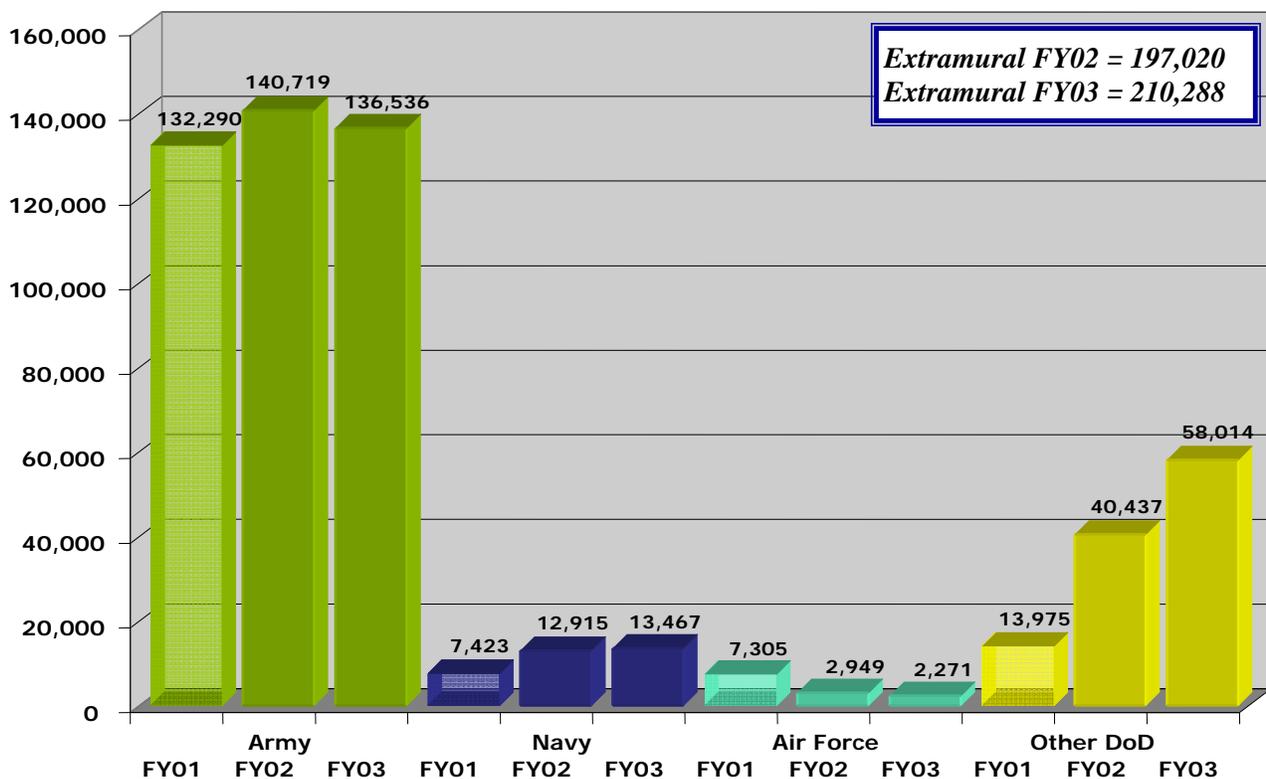


Figure 2-5 Extramural Animal Use by DoD Components for FY02–FY03 (FY01 for comparison)

Within the DoD, the Army is tasked with the greatest share of medical research conduct and oversight, using 62% of the total number of animals reported used by the DoD, 45% of the total number of intramural animals, and 82% of the total number of extramural animals. Overall, the Army’s animal use has remained steady at 62%–63% over the previous 3 years with both FY01 and FY03 totals at 209,000 and FY02 having risen to 224,000, an increase attributable to both intramural and extramural use.

Table 2-3 shows a list of CDMRP efforts with FY02 and/or FY03 funding greater than \$5 million, all of which are managed by the Army. Funding is dependent on yearly congressional appropriations. These programs used the majority 77%–82% of the Army’s extramural research animals and 31%–32% of the total DoD animal use in FY02/FY03. Among all of the Army’s extramural programs, the Breast Cancer Research Program utilized the largest number of animals. FY02 use (56,271) was similar to that in FY01 (57,382) while levels declined in FY03 (41,064). This program alone accounted for 16% of all animals used by the DoD in FY02.

Table 2-3 U.S. Army FY02–FY03 Congressionally Directed Medical Research Programs

Program	FY02	(\$ millions)	FY03
Alcoholism	5.6		4.2
Breast Cancer	150.0		216.0
Chronic Myelogenous Leukemia	5.0		4.3
Lung Cancer	3.5		9.0
Neurofibromatosis	21.0		20.0
Ovarian Cancer	10.2		10.0
Prostate Cancer	85.0		85.0
Prostate Disease	6.4		5.7
Peer Reviewed ¹	50.0		50.0

¹ The Peer Reviewed Medical Research Program addresses biomedical research with direct relevance to military health.

The Army is also the congressionally mandated lead agency for infectious disease and military dentistry research and the DoD Executive Agency for medical chemical and biological defense and nutrition studies. In FY03, Army research on infectious diseases, and chemical and biological defense used 36,830 and 40,020 animals, respectively.

Relative to FY01, Army animal use, both intramural and extramural, increased slightly in FY02 and fell in FY03. Overall, the Army has decreased its use of animals in research by 54% since FY94. As pointed out earlier (Section 2.2.1), the bulk of animal use under Army-administered programs derives from non-DoD mission-required activities directed by Congress. Discounting the considerable volume of CDMRP research, Army mission-related animal use has fallen by more than 60%.

The Navy used 18% of the total number of animals reported by the DoD in both FY01 and FY02 with this value declining to 14% in FY03. Since FY01, the largest change between FY01 and FY02 was a rise in extramural animal use of about 4,100 animals to 64,526 while the principal change between FY02 and FY03 was a reduction of nearly 17,000 intramural animals to an overall FY03 total of 48,163. Naval animal use climbed slightly (8%) in FY02 before declining 44% in FY03. Since FY94, Navy animal use has fluctuated between a low of 26,352 in FY97 to a peak in FY99 of 70,385 with the implementation of the FY96 Global Emerging Infections Surveillance and Response System (GEIS) program. The GEIS program was the result of Presidential Decision Directive NSTC-7 in June 1996 that directed all federal agencies to cooperate in surveillance and research on new infectious disease problems. Because of the DoD's wide-ranging assets for disease control, the mission of the DoD was expanded to support global surveillance, training, research, and response to emerging infectious diseases. The Navy conducts considerable infectious disease research on the pathogenesis and development of medical countermeasures to dengue viral illness and malaria, two mosquito-borne diseases.

Unlike the Army, where the predominate performer of animal research is extramural (e.g., CDMRP), the intramural programs of the Navy, Air Force, and other DoD Components exceed those of the extramural programs. The majority of animals (97% and 92% for FY02 and FY03, respectively) used by the Navy were used in medical research with 85% (53,106 [FY02]) and 77% (33,986 [FY03]) of this being research on infectious disease. Within the DoD, the Army and Navy share responsibility for research programs directed at the study of various infectious diseases likely to be encountered by troops deployed overseas. They also share responsibility for combat casualty research. The Navy employed 7,976 and 6,036 animals in that area in FY02 and FY03, respectively, with 88% used in extramural activities.

Within the DoD, the Air Force uses the fewest number of animals in that its mission is much more narrowly defined with respect to clinical and biological research. In FY02 and FY03, it used 1%–2% of the total number of animals reported used by the DoD, and even combining total animal use for FY02 and FY03, Air Force animal use was only 30% of that in FY94. The FY02 and FY03 totals comprise the lowest animal use numbers reported for any DoD Component since FY94. FY02 shows a 41% drop from the FY01 total contributed to by both extramural and intramural declines. Following that drop, FY02 and FY03 values remained relatively unchanged. The Air Force used 24% and 15% in clinical investigation projects in FY02 and FY03, respectively, and 59% and 47% of its animals in nonmedical research studies.

The contribution of other DoD Components, which include USUHS, DARPA, AFRRI, and AFIP, to the overall annual DoD total climbed from 14% in FY01 to 22% in FY03. While intramural use by these components fell from 19% to 12% in this period, extramural animal use increased in FY02, climbing from 13,975 in FY01 to 40,437 in FY02 and 58,014 in FY03 (28% of all FY03 DoD research). This trend was largely contributed to by a 2-year, 42,095 animal use increase in research conducted under DARPA programs. Overall, these DoD Components used the majority of their animals (62% and 77%, for FY02 and FY03, respectively) in medical research. Animal use in clinical research projects nearly doubled in FY02 to 14,178 before declining to 3,527 in FY03. Relative to FY94 (58,981), FY02 and FY03 numbers increased by 3% and 24%, respectively.

2.2.3 Animal Use by Species

The DoD uses three major classifications for reporting vertebrate animal use: Nonmammals, other mammals, and rodents (Figure 2-6). The use of rodents increased by 36,283 in FY02 from 295,449 in FY01. Total numbers of nonmammals declined over the 3-year period from 24,035 (FY01) to 10,997 (FY02) to 4,928 (FY03). The use of large numbers of fish in toxicity studies dominates this vertebrate category, accounting for this change. These numbers fell from 19,159 in FY01 to 3,296 in FY03.

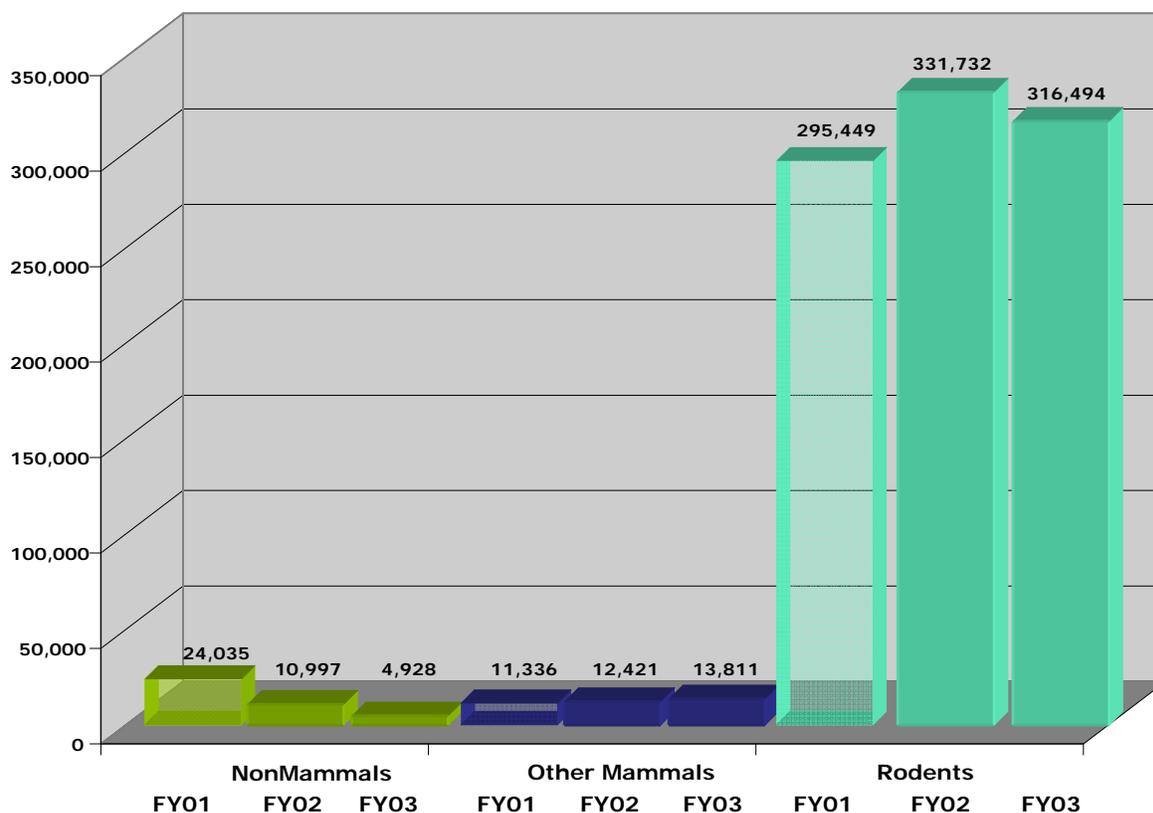


Figure 2-6 Nonmammals, Other Mammals, and Rodents for FY02–FY03 (FY01 for comparison)

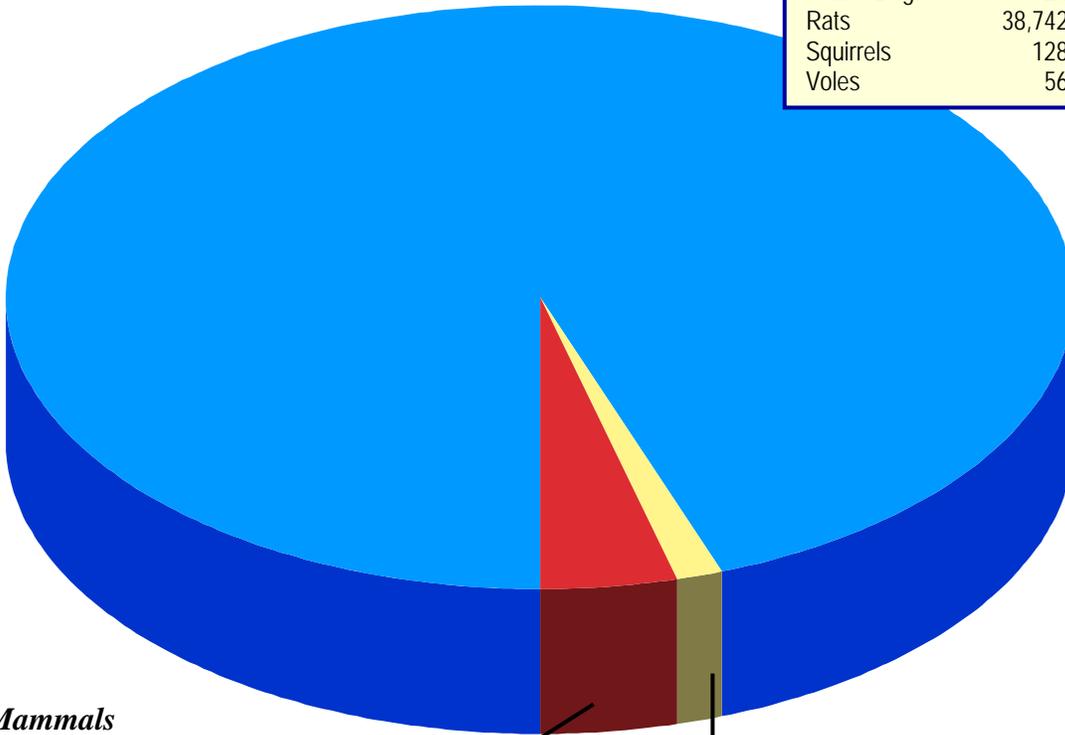
DoD animal use by species is presented in Figure 2-7. Figures 2-8 and 2-9 represent the intramural and extramural animal use by species for FY02 and FY03, respectively.

Considered the lowest phylogenetic species in preclinical research, mice and rats, which are both rodents, accounted for 90% and 91% of the DoD’s animal use in FY02 and FY03, respectively (see Figure 2-7). Mice are the predominant species used and generally account for most of the change in annual animal numbers. The use of other important rodent species such as rats and guinea pigs stayed relatively constant over the FY01 to FY03 period with rat and guinea pig use ranging between 36,714 (FY03) and 41,303 (FY01), and 6,676 (FY03) and 7,716 (FY02), respectively. By contrast, the use of hamsters increased slightly (6%) from FY01 to FY02 (2,597) and then increased 58% to 4,098 in FY03. Other rodent species are represented in much smaller numbers. The vast majority (96%) of animals species used by the DoD in both FY02 and FY03 were rodents, birds, amphibians, reptiles, and fish.

Total FY02 = 355,150
Total FY03 = 335,233

Rodents
FY02 – 331,732 (93.4%)
FY03 – 316,494 (94.4%)

Species	FY02	FY03
Chinchillas	206	262
Gerbils/Jirds	4	2
Groundhogs	4	0
Guinea Pigs	7,716	6,676
Hamsters	2,597	4,098
Mice	282,256	268,515
Prairie Dogs	23	0
Rats	38,742	36,714
Squirrels	128	199
Voles	56	28



Other Mammals
FY02 – 12,421 (3.5%)
FY03 – 13,811 (4.1%)

Species	FY02	FY03
Bats	11	465
Cats	34	31
Cattle	4	5
Dogs	451	254
Ferrets	94	110
Goats	3,076	2,681
Horses	29	25
Marine Mammals ¹	49	32
Mongoose	5	5
Nonhuman Primates	1,679	2,391
Pigs/Swine	3,055	3,734
Rabbits	3,631	3,883
Sheep	288	143
Shrews	15	52

¹Marine Mammals include:
FY02: Dolphins (33), Sea Lions (9), Seals (4), Whales (3)
FY03: Dolphins (16), Fur Seals (5), Sea Lions (7), Seals (2), Whales (2)

Nonmammals
FY02 – 10,997 (3.1%)
FY03 – 4,928 (1.5%)

Species	FY02	FY03
Amphibians	1,083	581
Avians ¹	1,596	672
Fish	8,211	3,296
Reptiles	104	379
Sharks	3	0

¹Avians include:
FY02: Birds (411), Chickens (1,132), Geese (35), Turkeys (18)
FY03: Birds (285), Chickens (311), Geese (37), Guinea Fowl (6), Turkeys (33)

Figure 2-7 Intramural and Extramural Animal Use by Species for FY02–FY03

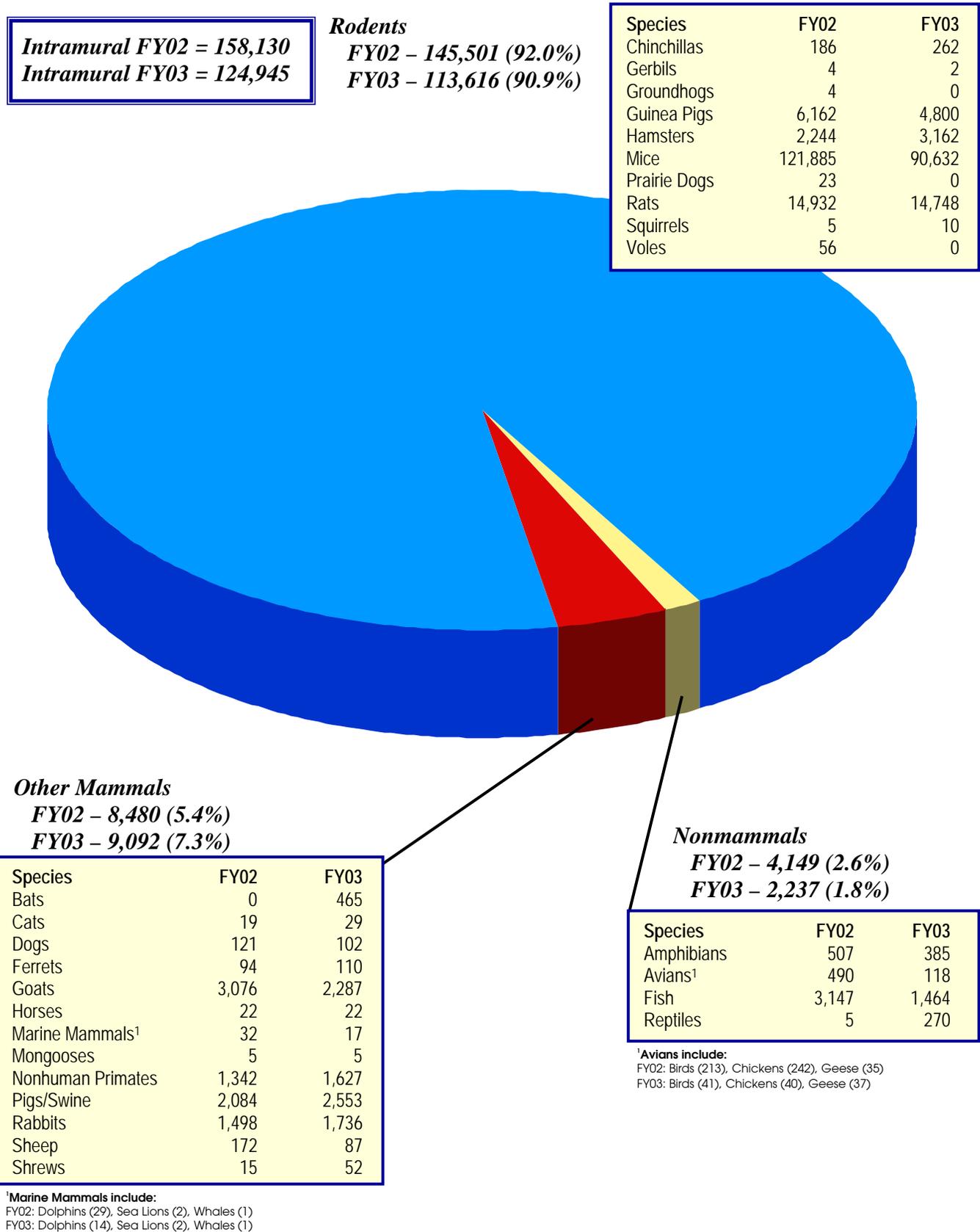
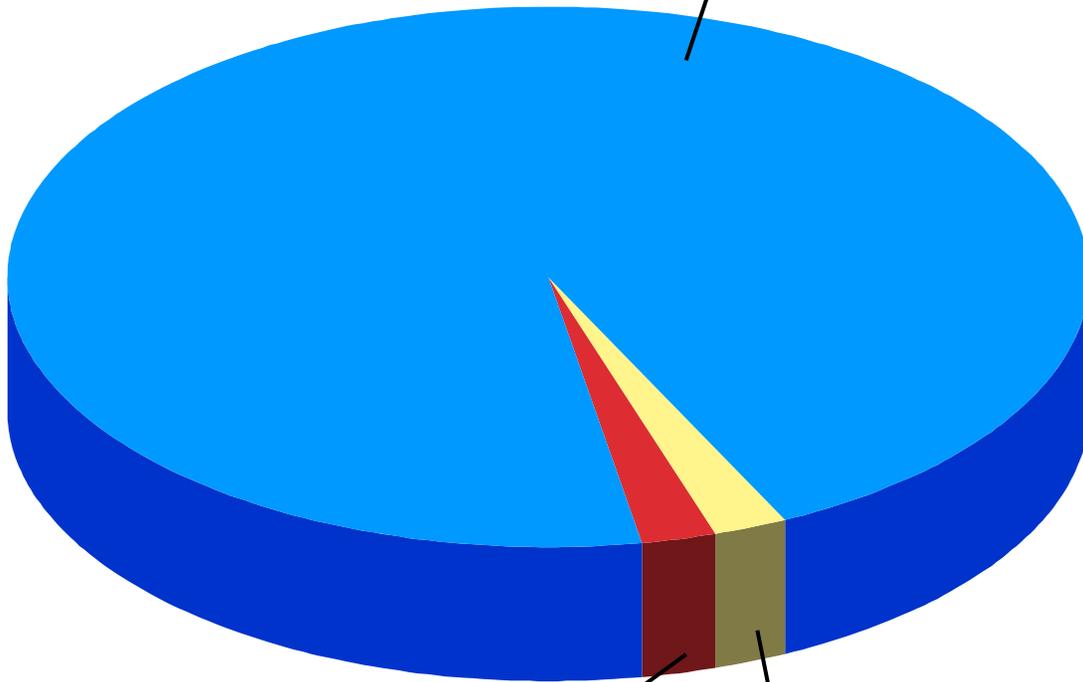


Figure 2-8 Intramural Animal Use by Species for FY02–FY03

Extramural FY02 = 197,020
Extramural FY03 = 210,288

Rodents
FY02 – 186,231 (94.5%)
FY03 – 202,878 (96.5%)

Species	FY02	FY03
Chinchillas	20	0
Guinea Pigs	1,554	1,876
Hamsters	353	936
Mice	160,371	177,883
Rats	23,810	21,966
Squirrels	123	189
Voles	0	28



Other Mammals
FY02 – 3,941 (2.0%)
FY03 – 4,719 (2.2%)

Species	FY02	FY03
Bats	11	0
Cats	15	2
Cattle	4	5
Dogs	330	152
Goats	0	394
Horses	7	3
Marine Mammals ¹	17	15
Nonhuman Primates	337	764
Pigs/Swine	971	1,181
Rabbits	2,133	2,147
Sheep	116	56

¹Marine Mammals include:
 FY02: Dolphins (8), Sea Lions (5), Seals (2), Whales (2)
 FY03: Dolphins (2), Fur Seals (5), Sea Lions (5), Seals (2), Whales (1)

Nonmammals
FY02 – 6,848 (3.5%)
FY03 – 2,691 (1.3%)

Species	FY02	FY03
Amphibians	576	196
Avians ¹	1,106	554
Fish	5,064	1,832
Reptiles	99	109
Sharks	3	0

¹Avians include:
 FY02: Birds (198), Chickens (890), Turkeys (18)
 FY03: Birds (244), Chickens (271), Guinea Fowl (6), Turkeys (33)

Figure 2-9 Extramural Animal Use by Species for FY02–FY03

Figure 2-10 represents the use of NHPs, dogs, and cats between FY94 and FY03. While the use of cats relative to FY01 showed a steady decline to 31 animals in FY03, the number of dogs used in research rose by 20% (74) in FY02, before falling 56% to a level of 254 in FY03. Preclinical studies resulted in a 42% increase in the use of NHPs in FY03 (712) relative to FY02. NHPs were primarily used in medical research (90%) in the areas of infectious disease (51%) and biological and chemical defense (25%). NHPs are unique in their ability to model human response to therapeutic compounds and are used in advanced preclinical research.

Over the FY02–FY03 period, 705 dogs were employed; the majority (76%) were used in medical research, notably in combat casualty care (35%), radiation research (25%), prostate cancer research (15%), and infectious disease research (10%). Other animals were used in neuroscience (8%) and medical training (13%). Dogs and humans are unique in their ability to develop prostate cancer, and the former are studied in extramural prostate cancer research. Dogs are also a unique model for leishmaniasis. Cats were almost entirely used in training (94%).

A cornerstone of the IACUC animal use review process is to ensure the use of the lowest possible animal species on the phylogenetic scale. While animal use protocol oversight requires the selection of the lowest possible species, differences in the physiology of mammals necessitate the use of animals such as dogs or NHPs in preclinical testing and in modeling humans. These types of studies can be expected to vary in their extent of overlap, resulting in peaks and troughs in the use of NHPs and dogs. Hence, while the use of dogs was the lowest ever in FY03, that year also saw the use of an unusually high number of NHPs.

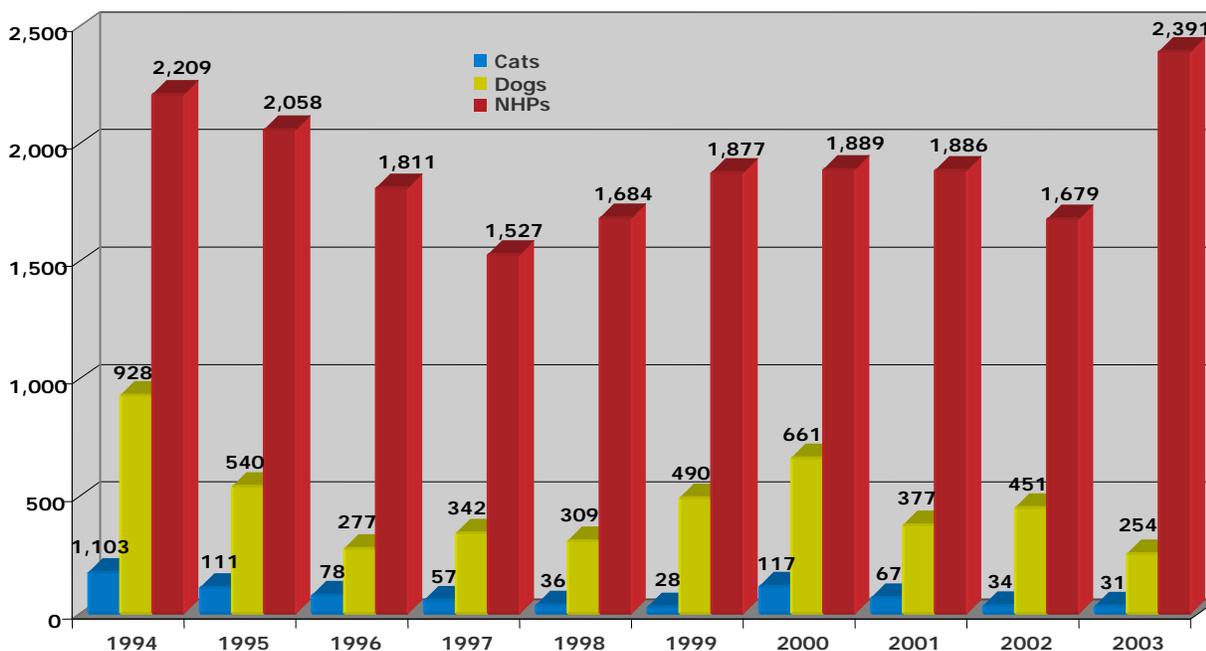


Figure 2-10 Use of Cats, Dogs, and NHPs by Fiscal Year

2.2.4 Animal Use by Animal Use Category

Total reported animal use in the DoD by animal use category, as defined in Table 2-1, is presented in Figure 2-11, with the intramural and extramural breakouts in Figures 2-12 and 2-13, respectively. The inset graph is an enlargement of categories wherein animal use is dwarfed by that in the medical research category (M). FY01 data are included to permit comparison.

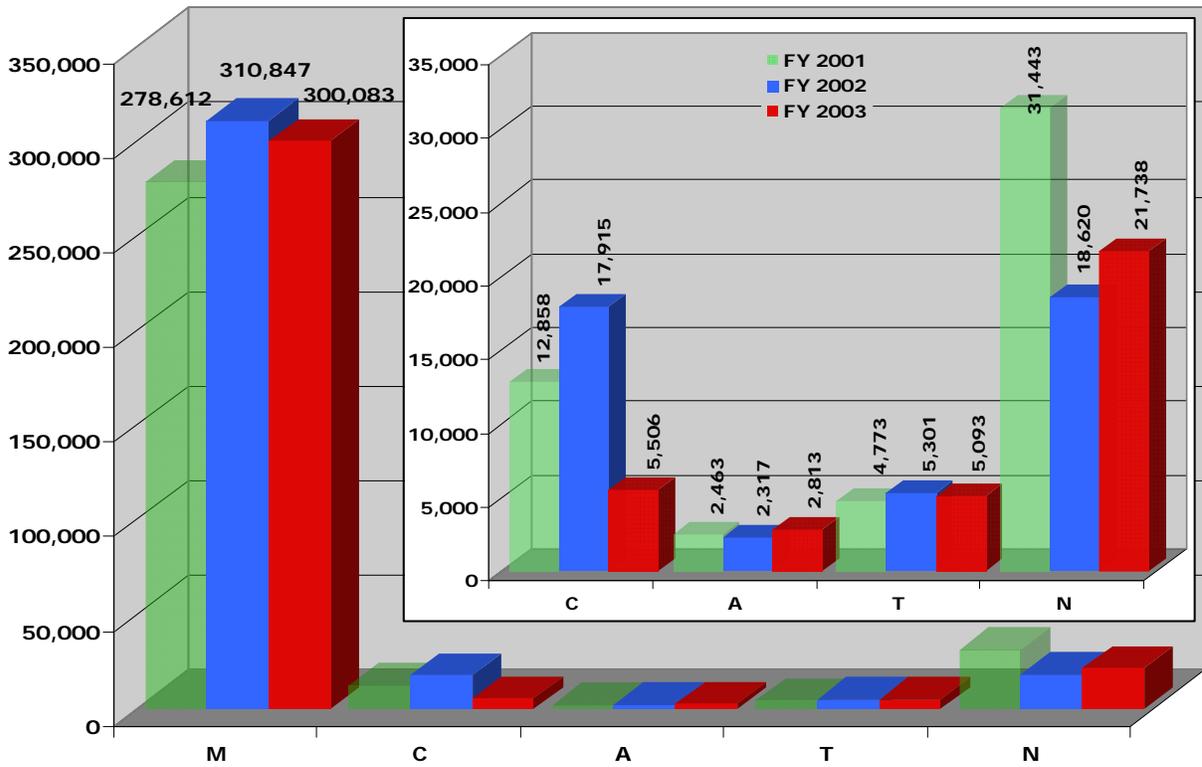


Figure 2-11 Intramural and Extramural Animal Use by Animal Use Category for FY02-FY03 (FY01 for comparison)

M-Medical RDT&E, C-Clinical Investigations, A-Adjuncts/Alternatives to Animal Studies, T-Training/Instructional, and N-Nonmedical RDT&E.

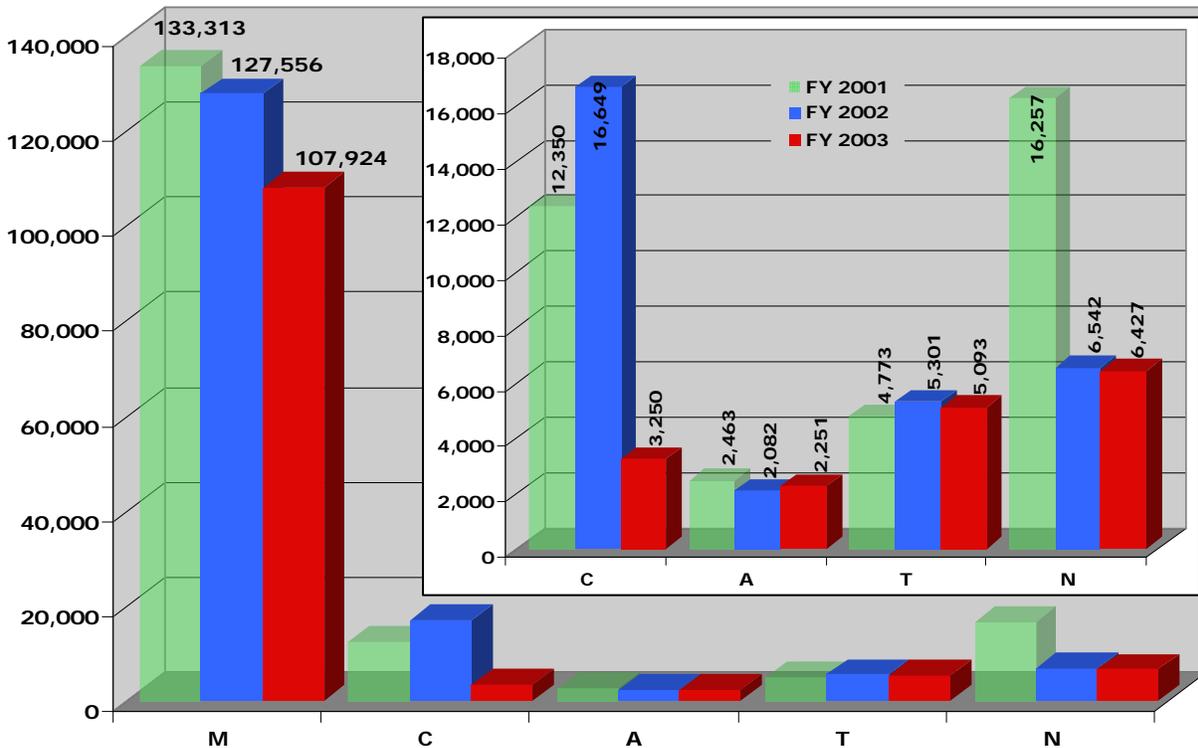


Figure 2-12 Intramural Animal Use by Animal Use Category for FY02-FY03 (FY01 for comparison)

M-Medical RDT&E, C-Clinical Investigations, A-Adjuncts/Alternatives to Animal Studies, T-Training/Instructional, and N-Nonmedical RDT&E. Totals may not add up to 100% due to rounding of calculations.

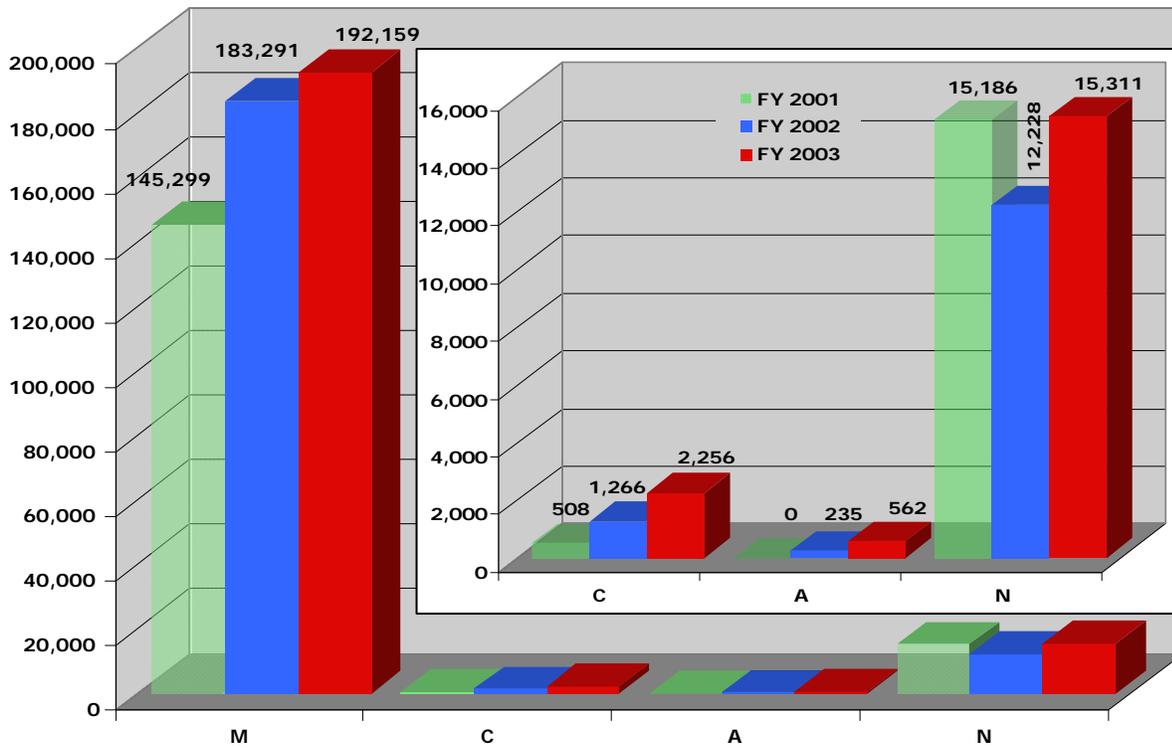


Figure 2-13 Extramural Animal Use by Animal Use Category for FY02–FY03 (FY01 for comparison)

M–Medical RDT&E, C–Clinical Investigations, A–Adjuncts/Alternatives to Animal Studies, T–Training/Instructional, and N–Nonmedical RDT&E.

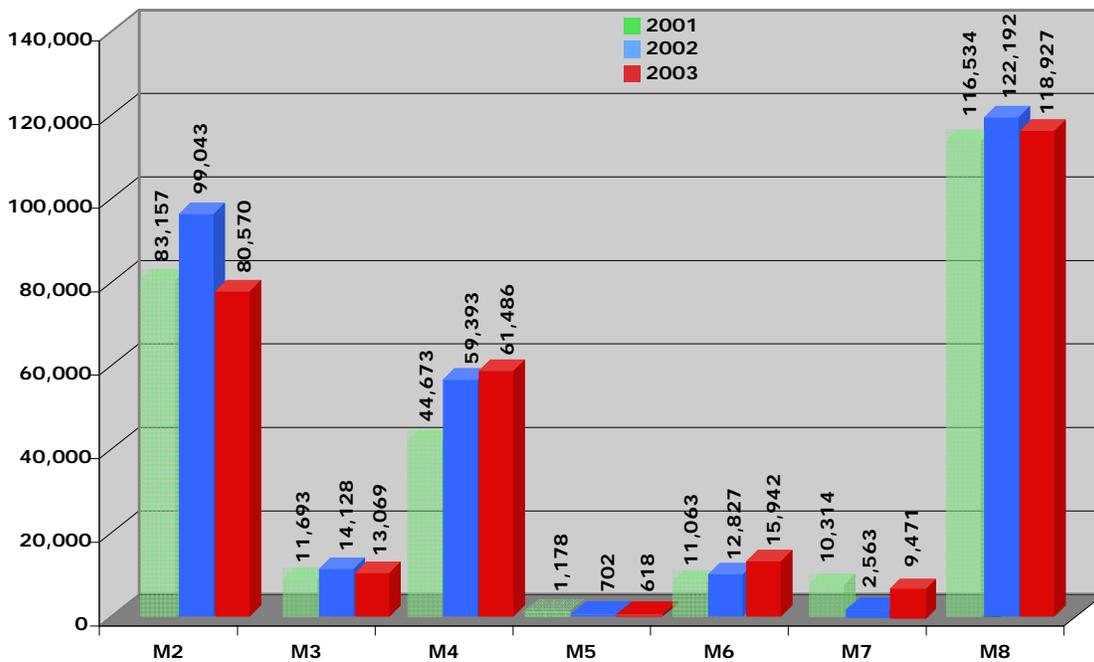


Figure 2-14 Animal Use by Subcategories of Medical Category RDT&E for FY02–FY03 (FY01 for comparison)

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 (M1, Military Dentistry, used no animals).

The DoD has a critical and challenging mission: To discover, design, and develop military countermeasures against threats to the health and survivability of military personnel. To meet this mission, 88%–90% of the animals used by the DoD in FY02–FY03 were in the medical category. Figure 2-14 shows the breakout by medical subcategories. No animals were used in subcategory M1, military dentistry. Overall, animal use levels among the different medical research category components remained steady between FY02 and FY03. In FY02 and FY03, 99,043 (32%) and 80,570 (27%), respectively, of the animals used in medical RDT&E were in the area of infectious diseases (M2). The primary thrust of this research is the development of preventive measures against infectious disease through the discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. During FY02 and FY03, the medical chemical defense program (M3) used 5% and 4% (14,128 and 13,069), and the medical biological defense program (M4) used 19% and 20% (59,393 and 61,486) of the medical category animals, respectively. The medical chemical defense program (M3) is conducted to develop improved pretreatments, therapeutics, and diagnostics to protect the warfighter from exposure to chemical warfare agents. The medical biological defense program (M4) is conducted to develop, demonstrate, and field new vaccines, drugs, and diagnostic kits for the prevention, treatment, and diagnosis of biological warfare agents such as anthrax. This research program protects members of the armed forces from the consequences of exposure to biological warfare agents and enhances their survivability. It has also assumed a central role in homeland defense and the development of countermeasures such as anthrax vaccines to terrorist threats.

Animal use for the medical RDT&E subcategory M5, which addresses the bioeffects of laser exposure, blast overpressure, operational stress, and occupational health protection, declined from 1,178 in FY01 to 702 in FY02. Animal use in M6 research increased 31% from 11,063 in FY01 to 15,942 in FY03. M6 research is directed at combat casualty care issues such as the development of blood substitutes and therapies for resuscitation, hemorrhage, shock, and tissue injury. M7, which addresses research into the effects of and treatment against exposure to ionizing radiation, showed the greatest change over the 3-year period with animal use declining by 74% in FY02 before returning to levels nearing 10,000 again in FY03.

M8 (Other Medical RDT&E) accounted for 39%–40% of the total medical category (Figure 2-14). The CDMRP (Table 2-3) used more than 110,000 animals per year in FY02 and FY03. These programs, which are managed by the Army, are primarily directed at cancer biology and account for 96% of M8 animals (Table 2-4), 35%–36% of the animals used in the medical RDT&E category (M), and 32% of the total DoD animals used in both years. These types of research programs can cause fluctuations in the total number of animals used from year to year depending on congressional funding levels and direction. Animal use in specific research areas of M8 are shown in Table 2-4.

Table 2-4 Breakout of Animals Used in “Other Medical RDT&E” (Subcategory M8) for FY02 and FY03

Subcategory	Animals Used	
	FY02	FY03
Bone Health Research	7,030	9,678
Breast Cancer Research	56,271	41,064
Defense Women's Health	381	0
Disaster Relief Emergency Services	176	0
Environmental Safety	73	365
Gulf War Illnesses	2,419	1,492
Medical Laser Research	455	438
Neurofibromatosis Research	4,469	6,852
Neurotoxin Research	9,462	10,460
Occupational Medicine	6	0
Ovarian Cancer Research	4,061	2,962
Prostate Cancer Research	29,137	29,926
Toxicology	436	1,078
Zoonosis	532	277
Other Uncategorized M8 RDT&E	7,284	14,335
Total FY Values	122,192	118,927

Clinical Investigations (Category C) accounted for approximately 5% (17,915) and 2% (5,506) of the animals used by the DoD in FY02 and FY03, respectively. Studies in this category address clinical medicine and surgical problems for the treatment of both diseases and combat casualties. While many of these activities address problems unique to the military, these clinical investigations also offer considerable benefit to the civilian sector.

Activities in the area of Adjuncts/Alternatives to Animal Studies (Category A) accounted for 2,317 and 2,813 animals in FY02 and FY03, respectively, illustrating the Department’s continuing efforts to ensure the health and welfare of the RDT&E animals under its care and promote research to develop alternatives to reduce, replace, and refine the use of animals in DoD research and training.

More than 5,000 animals per year were used by the DoD in FY02–FY03 in the training, education, and instruction of personnel (Training/Instructional, Category T). Under Category T, substantial efforts are directed to the training of field medics, surgeons, and veterinary personnel.

Nonmedical RDT&E animal use (Category N) accounted for slightly more than 5% (18,620) and 6% (21,738) of the total FY02 and FY03 animal use, respectively. Nonmedical RDT&E comprises a wide range of studies that are not generally directed at the solution of medical problems but are directed at the solution of militarily relevant problems through biological research. For example, there are a number of neurobiological studies addressing such areas as jet lag and sleep management. These included a large study of about 10,000 mice addressing the 24-hour biological clock that contributed substantially to the FY03 “Other DoD” total. In FY02, nonmedical toxicity studies employed nearly 6,000 fish, contributing substantially to FY02 numbers. Conceived in a deliberate effort to use the lowest species on the evolutionary scale, these fish studies employed 43%–45% of the total Category N animal use in FY01–FY02.

2.2.5 Animal Use by USDA Pain Category

Total reported animal use in the DoD by USDA pain category is presented in Figure 2-15, with the intramural and extramural breakouts in Figures 2-16 and 2-17, respectively.

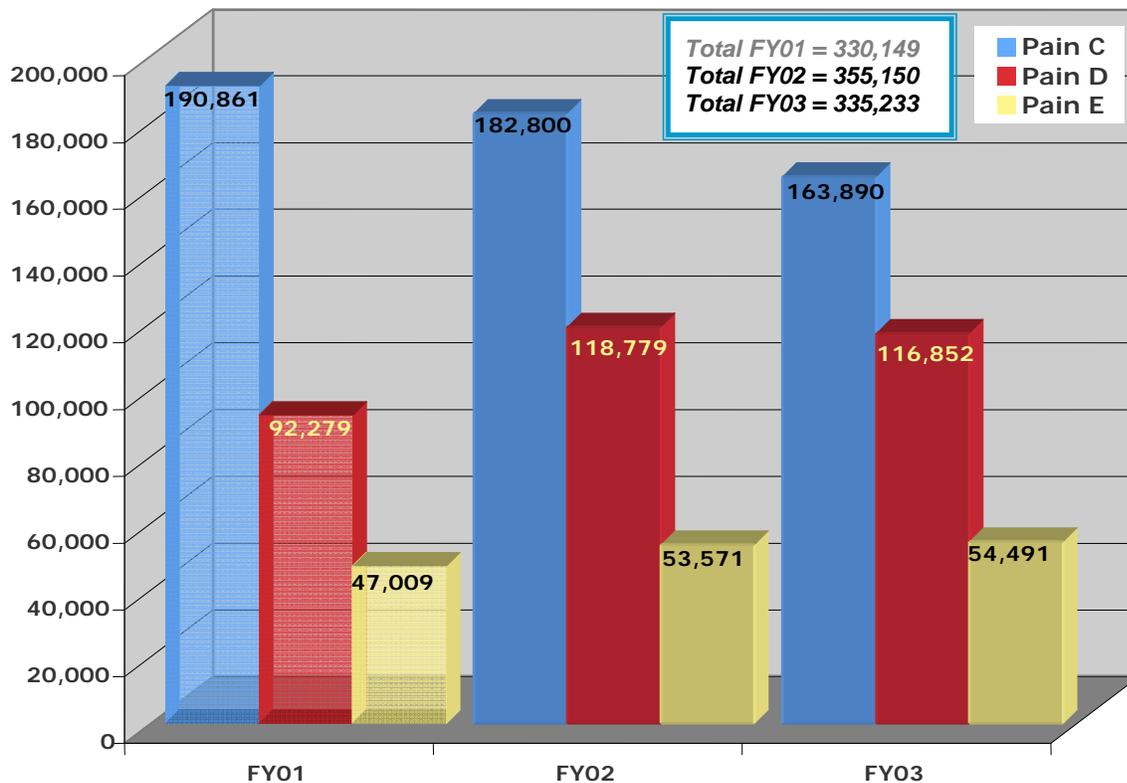


Figure 2-15 Intramural and Extramural Animal Use by USDA Pain Category for FY02–FY03 (FY01 for comparison)

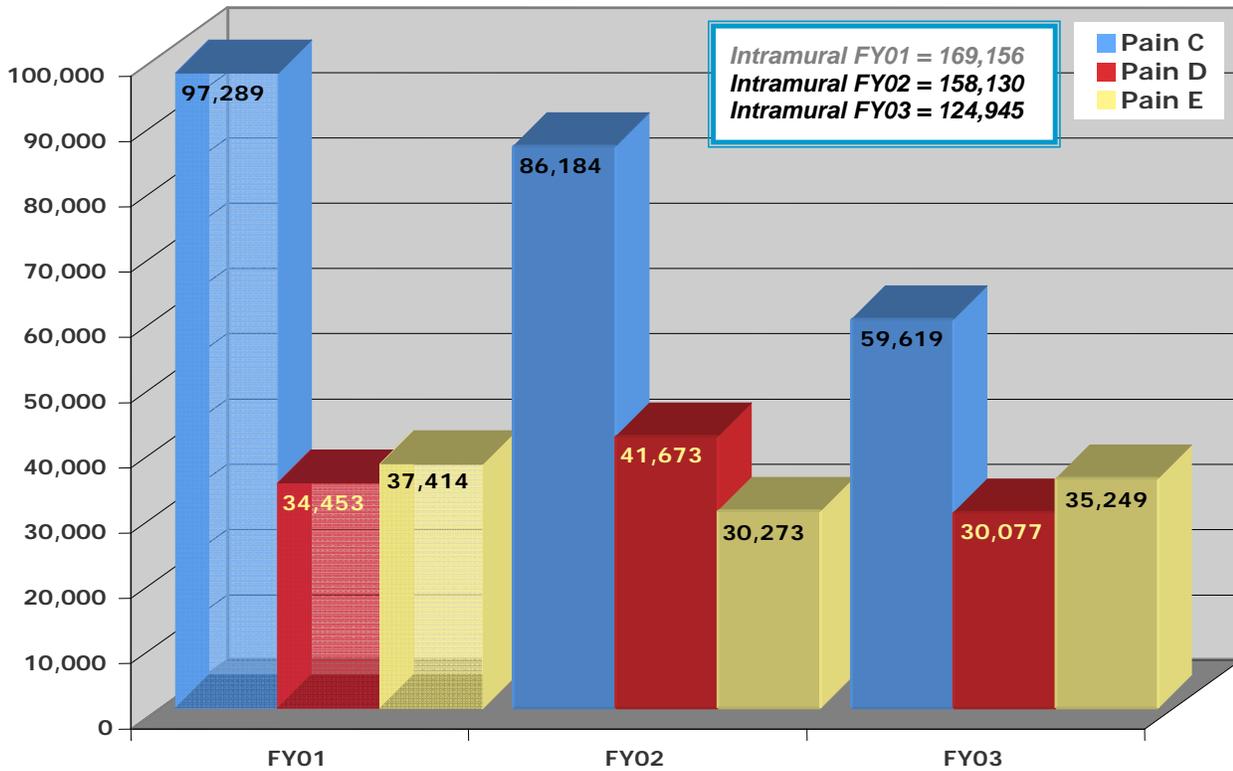


Figure 2-16 Intramural Animal Use by USDA Pain Category for FY02–FY03 (FY01 for comparison)

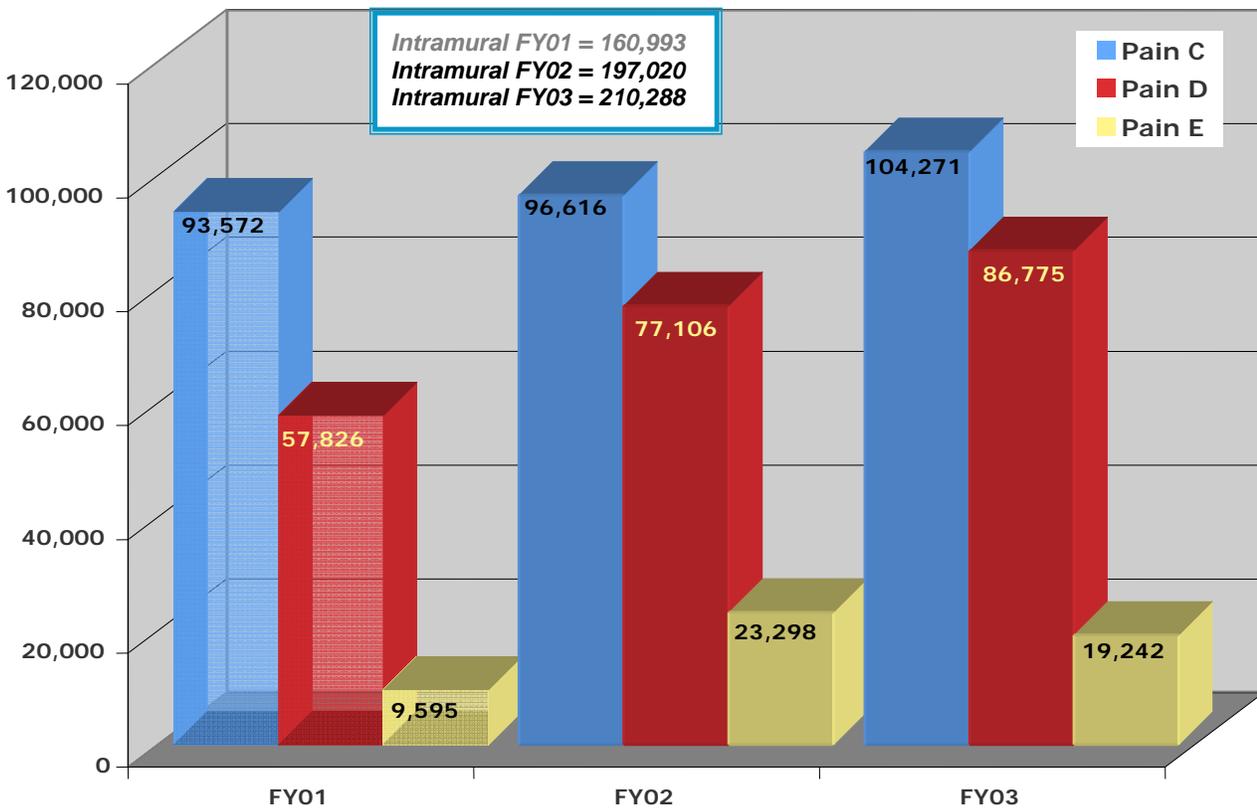


Figure 2-17 Extramural Animal Use by USDA Pain Category for FY02–FY03 (FY01 for comparison)

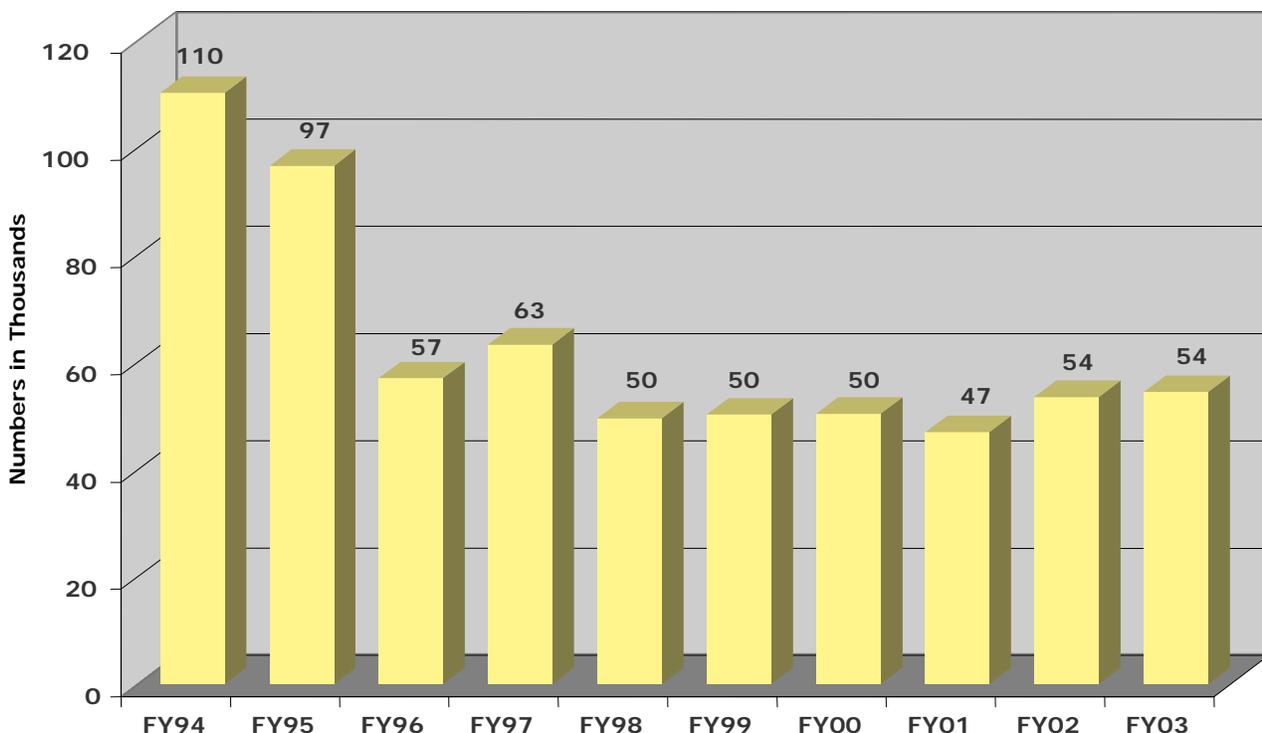


Figure 2-18 USDA Pain Category E by Fiscal Year

The majority (84%–85%) of FY02–FY03 DoD-supported research employing any species of animal was considered not painful to the animals involved. Half of all animals were not exposed to or involved in any potentially painful procedures (USDA Pain Category C). Between 33%–35% of all animals were given anesthesia or pain-relieving drugs to prevent pain or distress (USDA Pain Category D). In 15%–16% of all animals used, anesthetics or analgesics were not used because they would have interfered with the validity of the results of experiments (USDA Pain Category E). Since FY94, the use of animals in Pain Category E decreased sharply and has remained relatively steady over an 8-year period (Figure 2-18).

The words “potentially painful” reflect the fact that a Pain Category E classification is applied to any protocol wherein pain might be realized or cannot otherwise be fully assessed. For example, per USDA policy, a Pain Category E designation was used for more than 2,600 fish subjected to low-level toxicity studies in FY02. Although they showed no signs of distress during the study, they must be assigned to this category for lack of an effective way to monitor any discomfort. It should be emphasized that every effort is made to reduce or eliminate unnecessary suffering by animals involved in all studies.

Figure 2-19 shows the numbers of animals used in Pain Category E by the animal use category. In FY03, 96% of the animals reported in USDA Pain Category E were used in medical studies (Category M). Of these, 69% of the animals were used in research on infectious disease (M2), medical chemical defense (M3), and medical biological defense (M4). Overlap between inflammatory, pain, and immune response mechanisms may preclude the use of pain alleviation in achieving meaningful research results. Ionizing radiation studies (M7) employed 11% of all FY03 Pain Category E animals. The number of animals in Pain Category E was much lower in the remaining M subcategories and in Categories A, C, and N. There were no animals subjected to unalleviated pain in Category T.

Typically, a majority (94% in FY03) of the animals used in potentially painful experiments were rodents. Other mammals accounted for 1.5% of animals in this pain category in FY03.

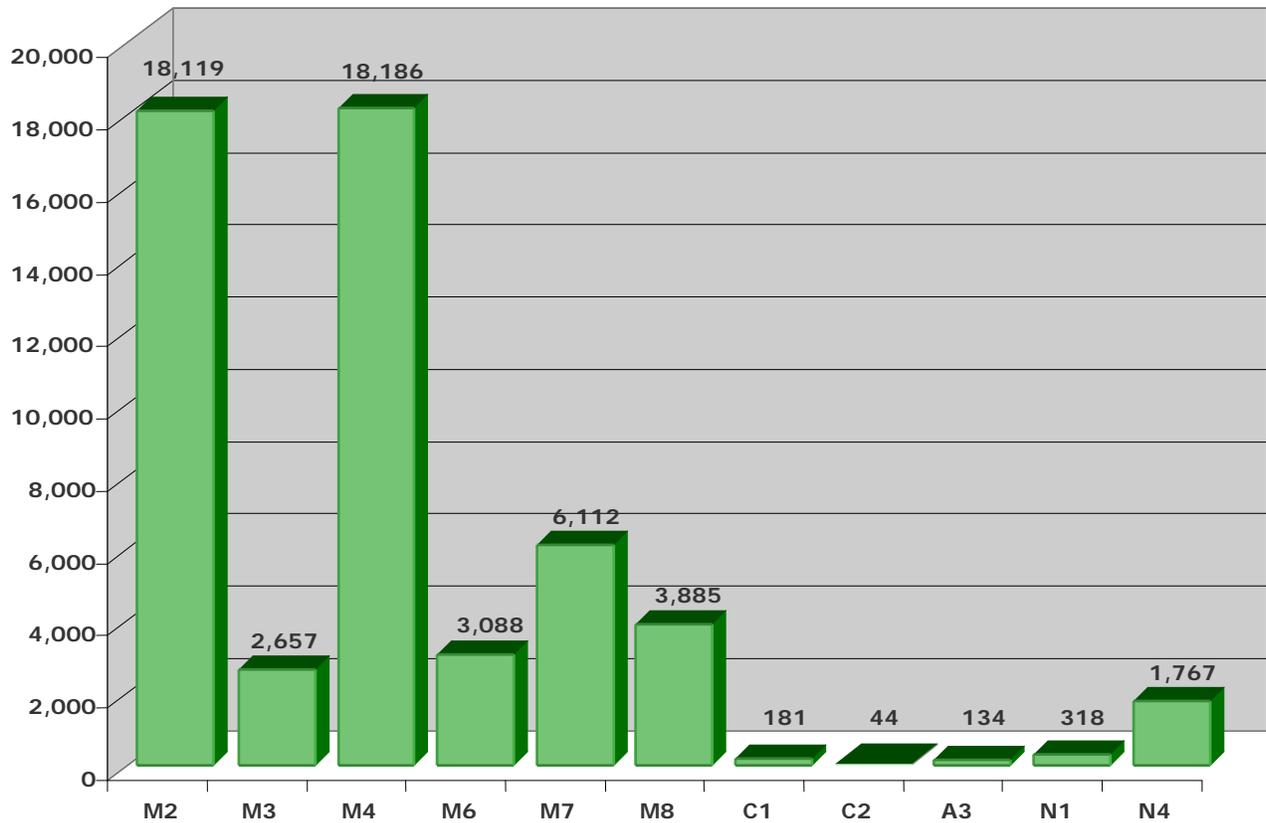


Figure 2-19 Number of USDA Pain Category E Animals by Animal Use Category

The DoD clearly has a diverse, unique, and demanding RDT&E mission that provides the context for Pain Category E research. 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 altitude pressure, and gravitational forces are threats to service men and women. The DoD must provide acceptable protection against these threats and many others, and the animals reported in USDA Pain Category E were used in research designed to find ways to protect service men and women from the threats encountered over the course of performing their missions.

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SECTION 3

DoD INITIATIVES TO PROMOTE ALTERNATIVE METHODS THAT REPLACE, REDUCE, AND REFINES 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 DoD advocates a fourth R, “responsibility,” for implementing these alternative methods.

Replacement

The replacement alternative addresses supplanting animal use with nonliving systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. It includes the elimination of animal use altogether, generally by adopting in vitro or theoretical model study systems. Replacement also includes the substitution of species that are higher on the phylogenetic scale with those that are lower.

Reduction

Reduction is the use of fewer animals without loss of scientific test validity. Decreasing the number of animal subjects through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

Refinement

Refinement is a procedure or measure taken to eliminate or minimize pain or distress in the animal(s) or enhance well-being while maintaining or improving the quality/quantity of research data collected. Examples of refinement include, but are not limited to, the use of analgesia to decrease pain or distress; the use of remote telemetry, which decreases the distress of restraint; the use of adjusted early experimental endpoints; and the improvement of quality of life in animal housing.

Responsibility

The DoD has taken responsibility for implementing animal use alternatives. It is reflected by the Department’s efforts to replace, reduce, and refine animal use in the context of ensuring scientific validity, study needs, and animal well-being. 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 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 lists included in this section are not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD’s research projects is extensive. Rather, they illustrate the scope, diversity, and spirit of the DoD’s four Rs initiative. This section will demonstrate a broad-based movement toward the use of biotechnology and other innovative adjuncts to replace and reduce animal use as well as refinement in methods used in essential animal studies.

3.1 DoD INITIATIVES TO PROMOTE ANIMAL ALTERNATIVES

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will replace, reduce, and refine 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.

3.1.1 DoD-funded Research, Conferences, and Workshops to Develop Alternatives to Animal Use

Over the years, the DoD has continued to seek alternatives to animal use through a research objective initiated in FY93 entitled “Reducing Reliance on the Use of Animals in Research and Improving Experimental Conditions

Using Animals.” The purpose of this objective plan has been to conduct basic research to develop new technologies to incrementally reduce future reliance on research animals. In FY02-FY03, chemical and biological defense projects totaling over 1.3 million dollars were implemented in accordance with the objectives of this initiative.

Since 1990, the DoD has regularly supported progress toward implementing alternatives to animal use by sponsoring major meetings and conferences on the subject. The DoD periodically cosponsors international meetings on alternatives to animal testing. These conferences have been sponsored by the U.S. Army Soldier and Biological/Chemical Command and such prestigious cosponsors as the National Institute of Environmental Health Sciences (NIEHS), the U.S. Army Medical Research Institute of Chemical Defense, the U.S. Army Center for Health Promotion and Preventive Medicine, the U.S. Navy, the U.S. Air Force, Xenogen Corporation, the Gillette Company, the Humane Society of the United States, DermTech International, Interagency Committee on Neurotoxicology, Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Capital Area Chapter of the Society of Toxicology, and the Association of Government Toxicologists. These international meetings serve as a scientific forum to exchange research papers and poster presentations. Proceedings of symposia are available through the Defense Technical Information Center. The DoD was a substantial contributor to the 2003 publication of a 40 chapter book concisely discussing the application of state-of-the-art methods and cutting-edge research related to developing and validating alternatives to animal testing. Bringing together the contributions of over 125 scientists from industry, government, and academia, *Alternative Toxicological Methods for the New Millenium*, edited by Sidney Katz and Harry Salem (CRC Press), explores the development and validation of replacement, reduction, and refinement alternatives (the 3Rs) to animal testing.

3.1.2 DoD Support for the National Research Council’s Institute of Laboratory Animal Research Educational Programs

The Department’s priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in financial support of the Institute of Laboratory Animal Research (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is the development of institutional training materials, educational courses, and publications in support of the Department’s laboratory animal care and use programs. 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. ILAR information and programs have generated strong animal alternative provisions for both civilian and military-specific research opportunities.

3.1.3 DoD Participation in Federal Animal Alternative Programs

The National Institutes of Health Revitalization Act of 1993 (PL No. 103-43, Section 1301) directed the NIEHS of the NIH to establish an Applied Toxicological Research and Testing Program, which represents the NIEHS component of the National Toxicology Program. The Act further directed the NIEHS to “(a) establish criteria for the validation and regulatory acceptance of alternative testing methods and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use.” To fulfill this mandate, an ad hoc ICCVAM was established in 1994 by the NIEHS. In 2000, PL 106-545, the ICCVAM Authorization Act of 2000, established ICCVAM as a permanent committee. The mission of ICCVAM is to coordinate issues throughout the federal government that relate to the development, validation, acceptance, and harmonization of toxicological test methods. The ICCVAM is responsible for the coordination of the development and review of various alternative toxicological methods. The ICCVAM must also facilitate communication among all stakeholders in the development and review process of alternative methods. The ICCVAM evaluates proposals for alternative test methods and recommends further research. It comprises 47 members representing 15 different U.S. federal agencies. Members serve as points of contact and as sources to identify technical experts from their agencies to serve on specific topical working groups. Recommendations regarding the usefulness of test methods provided by ICCVAM enable U.S. federal agencies to assess risks entailed by various test methods and make regulatory decisions. The ICCVAM determines which assays warrant peer review, forms working groups, and supports test method workshops. When members of the ICCVAM agree that an alternative method merits investigation, a working group is assembled. The working group in turn determines whether sufficient information exists for the assembly of either a peer review or a test method workshop. Results generated by

ICCVAM's working groups may be used to recommend U.S. federal regulations and/or guidelines for research. More information on ICCVAM can be found at <http://iccvam.niehs.nih.gov/>.

3.2 DOD EXPERTISE AND TRAINING PROGRAMS THAT PROMOTE ANIMAL ALTERNATIVES

3.2.1 Professional Veterinary Training in Laboratory Animal Medicine

In FY02–FY03, the DoD's veterinary training programs yielded 7 residency graduates and 6 board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM). The DoD sponsors formal postdoctoral training programs for veterinarians in LAM, including a nationally recognized, 4-year program consisting of 2 years of residency training and 2 years of practical experience, culminating in specialty board eligibility for certification by the ACLAM. In August 1995, the DoD began a formal postgraduate master's of public health in LAM at the USUHS.

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, such as the American Association for Laboratory Animal Science (AALAS), the American Society of Laboratory Animal Practitioners, and ACLAM were formally trained in, or closely associated with, DoD LAM training programs. This strength in LAM expertise strongly enhances both animal care and use and animal alternative programs.

3.2.2 Veterinary Staff Expertise and Assistance Visits

The DoD Component oversight offices all have credentialed LAM veterinarians who act as advisors to military commanders on issues related to animal welfare and alternatives to animal use, and provide oversight to the command's animal care and use programs. These veterinarians make periodic assistance visits to the laboratories to address the consideration of animal use alternatives.

LAM veterinarians are also assigned to DoD research institutions and provide expertise in many ways. An important responsibility of LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed.

3.2.3 AALAS Technician and Laboratory Animal Science Training

A number of DoD research facilities sponsor training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Workshop present formal training and information on alternatives to animal use. In addition, the Walter Reed Army Institute of Research (WRAIR) offers quarterly workshops on ethical and administrative issues in animal use. Both the AALAS technicians' course and WRAIR workshop curriculum include formal training and information on animal use alternatives.

3.2.4 DoD Publications of Animal Use Alternatives

DoD experts have published several documents on animal use alternatives in toxicology. Most recently, a book entitled *Alternative Toxicological Methods* (CRC Press, 2003) was supported by the Army and cowritten by a senior Army scientist.

3.3 DOD IMPLEMENTATION OF GENERAL ALTERNATIVES TO ANIMAL USE

DoD animal use alternatives are categorized as "general" and "specific." General alternatives are frequently implemented in many different DoD programs. They include some standard practices, such as the statistical minimization of animal use for each protocol and other practices that are strongly encouraged through the IACUC review process. Specific alternatives are more unique than their general counterparts. They could be relevant only to a single protocol or to a single facility and are discussed in Section 3.4.

3.3.1 Research Protocol and IACUC 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 constituting and operating 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 nonanimal alternatives be considered. It states, “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. Instructions for the Standard Protocol Format state, “investigators should use the least sentient species that will permit the attainment of research objectives.” In addition, investigators are required to provide a short description of the features of the proposal that may qualify the study as one that replaces, reduces, or refines the use of animals. The [DoD 1995 Policy Memorandum](#) requires that extramural contractor proposals utilizing animals in research, education, testing, or training include all of the information contained in the DoD Standard Protocol Format, thereby requiring the alternatives information.

3.3.2 Policies Emphasizing Refinement

In addition to the implementation of alternatives in research protocols, the DoD has established policies specific to the refinement of animal use in general animal housing and maintenance. This policy allows for flexibility and creativity for improving conditions for laboratory animals. Environmental enrichment can include the provision of toys, increased housing space, or social housing strategies. For example, WRAIR was one of the first institutions to establish a policy that mandates consideration of environmental enrichment for research animals. In FY03, animals assigned to 590 projects at WRAIR benefited from the implementation of environmental enrichment strategies.

The list below provides brief examples that are representative of the general alternative methods used by DoD facilities during FY02–FY03.

Replacement

- During the review process, all potential methods of adequately answering a research objective are reviewed before employing an animal model.
- The evaluation process also considers the selection of a particular animal type; species lower on the phylogenetic scale are considered and used if their selection permits attainment of the research objectives.
- Hands-on, nonanimal training aids are used to replace the use of live animals.
- Computer simulations can partially or completely replace live animals.

Reduction

- Animal use protocols are subject to review by a biostatistician who addresses the animal used, study design, and statistical evaluation packages, and ensures that the minimum number of animals will be used to meet the specific scientific objectives.
- Pilot studies are used to refine techniques and define the animal model so that animal use can be kept to the minimum required for statistical significance.
- Sharing of animal tissues among investigators reduces animal use.
- Iterations of the experiments are combined when possible to reduce the number of control animals used.
- Collaboration between DoD investigators or instructors allows for a single animal to be used in multiple training or research procedures and the sharing of control group information, resulting in an overall reduction in the number of animals used.
- Several types of data are collected simultaneously.
- Training sessions are designed to use the highest practical student-to-animal ratio.
- When possible, animals serve as their own controls.
- Studies are deliberately phased so they continue only if warranted.

Refinement

- Parameters developed for early or alternative endpoints are used as experimental endpoints when possible.
- Animals are anesthetized before euthanasia to decrease stress.
- Moribund animals are humanely euthanized to prevent unnecessary pain or distress.
- Utilizing the environmental enrichment strategy, animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nest boxes and toys).

- Animal-handling skills and clinical techniques are taught to animal technicians, investigators, and research assistants to increase or ensure that a proper skill level is attained prior to the start of a protocol.
- All advanced trauma life support training laboratory procedures are performed while animals are under general anesthesia, and they are euthanized without regaining consciousness.

3.4 DOD IMPLEMENTATION OF SPECIFIC ALTERNATIVES TO ANIMAL USE

FY02–FY03 DoD research shows that DoD organizations are actively involved in the development of alternatives to animal use. These developments have occurred through research specifically designed to produce alternatives and improve experimental techniques. Whenever possible, DoD investigators attempt to develop state-of-the-art, scientifically relevant, and reliable experimental procedures that can be performed without the use of animals. In addition, in cases where animal models cannot be completely replaced, investigators and veterinary staffs work diligently to develop refinement techniques to minimize animal pain and distress and improve the quality and quantity of data through the use of technology. The DoD is very active in the development of alternatives to the use of animals in research.

3.4.1 Specific Alternatives Employed during FY02–FY03

The following list provides brief examples that are representative of the specific alternative methods reported used by DoD facilities during FY02–FY03.

Replacement

Replacement Using In Vitro Cell Cultures:

- Antiviral drugs were initially tested in vitro for their ability to inhibit viral replication. Drugs that did not show activity in vitro were not tested in mice.
- The biological activity of purified antigens was assessed using tissue culture techniques instead of animals.
- Monoclonal antibodies were obtained using tissue culture instead of mice.
- In vitro studies of seizures were performed using cultured neurons.
- Cell culture assays were used to measure antibody levels instead of combining serum with toxin and injecting the mixture back into mice to determine if they would be protected from toxin challenge.
- Anticancer drugs were tested in cell culture to reduce the number of animals needed for testing.

Replacement Using Nonmammalian Species or Species Lower on the Phylogenetic Scale:

- Prior to using primates, mice were used to model vaccination strategies with regard to dosing, injection strategies, and formulation.
- Frogs replaced mammals in the study of effects of toxicants on the development of reproductive organs.
- Japanese medaka fish, instead of rodents, were used in studies of toxicity mechanisms.
- Monkeys most closely model human intoxication with Staphylococcal Enterotoxin B toxin. However, an actinomycin D mouse model of staphylococcal enterotoxin B intoxication was developed and has been demonstrated to be similar to monkeys with respect to histopathologic features.
- A mouse model for TSST-1 *Staphylococcus* was developed to replace the rabbit, which is the standard model for TSST-1 research.
- A noninvasive snake model was developed to replace the rhesus monkey in retinal research.

Biochemical/Physical Methods and Other Technologies:

- Antibody yields expected from the milk of a cow are estimated to equate to that produced by 10,000 mice—a simple and nonpainful procedure replacing the use of a large number of mice. Goats are similarly employed.
- Pig's feet and oranges purchased from a local grocery store are used to teach skin biopsy, suturing techniques, and intraosseous fluid administration, replacing live animal use.
- Computer simulations such as the Simulab Trauma Man mannequin partially or completely replaced live animals in advanced trauma life support training programs.
- Resus-a-pup mechanical models were used to teach canine CPR, eliminating the use of live dogs.

Reduction

Substitution of Computer Simulation, Models, or Other Technologies:

- Intubation was practiced using endotracheal models prior to utilizing anesthesia-teaching protocols.
- Koken and PVC rat models familiarized students with equipment, procedures, and anatomy before they were allowed to work with live animals.
- Membrane feeding techniques were employed to reduce the number of animals necessary to maintain mosquito colonies.
- A study design employing multiple behavioral tasks required fewer animals to reach statistical significance as compared to separate groups being employed for each behavioral procedure.
- Some ophthalmology training employed bovine eyes from a local slaughterhouse, eliminating the need for rabbits.
- Computer models are used to evaluate protocols and eliminate those unlikely to produce desired results.
- Installation of advanced caging systems and vivarium technology reduced the number of animals required to monitor colony infections.
- Pediatric mannequins were used in training to give students some experience in establishing airways before using animals, reducing the likelihood for injury and the number of animals required.
- Artificial rat models helped surgical residents develop their microvascular and microneural surgical skills before they use live animals.
- To reduce the numbers of animals employed in testing vaccine constructs, all plasmids were first tested in vitro for expression of the candidate vaccine product.
- In vitro cell culture-based protocols were developed to allow for activity screening in vaccine development and to reduce the number of mice required for testing.
- Physiologically based pharmacokinetic computer modeling to investigate toxicity reduced the number of animals needed to complete toxicological assessments.
- In dental readiness training, mannequins were initially used to give students experience in establishing airways, requiring fewer pigs.
- Using primary neuronal cell cultures markedly reduced the total number of rats required for neuroprotection experiments because multiple culture dishes can be prepared from single rat embryos.
- A computerized system was developed for data acquisition and the control of the hemorrhage protocol, allowing for the collection of large amounts of data with reduced error and animal use.
- Mosquito colonies were successfully adapted to membrane feeding. The technique, now fully adopted, eliminates direct feeding on rabbits and terminates another protocol using direct feeding on mice.

Experimental Strategies:

- Limbs and body parts harvested from protocols in which animals were euthanized were subsequently used in the training of intubation, injury care, dental management, and pathology.
- Prescreening in mice yielded dose-ranging data that reduced the subsequent number of NHPs required for *Brucella* vaccine testing.
- The number of monkeys is being reduced through the use of in vitro viral tissue culture infectivity titers instead of in vivo titers from monkeys.
- Control group data were generated once and subsequently used in multiple experiments.
- Precision-controlled peristaltic pumps increased experimental reproducibility and reduced the number of animals required in experimentation.
- Use of a confocal scanning laser ophthalmoscope for retinal imaging is noninvasive and pain free and reduced the need for prolonged anesthesia in producing localized, laser-induced retinal lesions and the need for sacrificing subjects in observing the effects of long-term laser exposures.

Refinement

Reducing Pain and Distress:

- Chinchillas were behaviorally conditioned to reduce separation anxiety from their conspecifics.
- Having discovered blood pressure oscillation to be the earliest marker of light anesthesia, occurring about 5–10 minutes before the return of corneal reflexes and pain response, researchers now routinely use it as a signal for giving additional anesthesia.
- A hanging, three-dimensional, environmental enrichment center for a cat colony was designed and constructed. This series of baskets, ledges, hammocks, and toys added mental, physical, and visual variation to the cat room while providing horizontal resting places required by law.
- Sedation procedures for rhesus monkeys were employed that reduce/eliminate animal stress in a procedure that would normally require secure confinement for phlebotomy and immunization procedures.
- Animals were acclimated and trained to be led by a halter or collar to minimize pre-phlebotomy stress.
- Primates were monitored using noninvasive technologies including continuous subcutaneous temperature recording and ultrasonography to measure organ involvement.
- Rats were trained to use shaded restraint devices, from which blood and urine samples were collected via indwelling catheters, eliminating the need for repeated sampling and reducing distress.
- Surface and subdermal electrodes were used so that rats would not have to undergo surgery or live with electrodes implanted in their heads for long-term electrophysiological follow-up after nontoxic exposure to a chemical agent.
- Radiotelemetry was used to monitor physiological parameters in a number of projects involving rats and NHPs, reducing stress.
- An alternative surgical approach in vascular surgery was developed for rabbits, reducing the risks of nerve and muscle injury.
- Use of an isolated organ eliminated the discomfort, pain, and suffering that would occur if transfusion-related lung injury was induced in the whole animal to assess an in vivo end point.
- Animals were euthanized at the first signs of infection to reduce prolonged suffering.

Increased Training for Research Personnel to Improve Skills:

- Development of training programs to teach research personnel the technical skills necessary to properly manage and humanely handle NHPs during research experiments.
- Instruction in the care, handling, and management of rodents and lagomorphs.
- Development of veterinary techniques training programs for personnel utilizing various laboratory animal species, which will result in better animal handling.
- Training in surgical and aseptic techniques, which results in shorter surgery duration, less tissue trauma, and decreased postoperative complications.

3.4.2 Alternatives Undergoing Development during FY02–FY03

As an ongoing process, the DoD is continuously developing alternatives. Below are examples of alternatives that were reported as currently in development by the DoD during FY02–FY03. This is only a sample of the alternatives being developed this year.

Replacement

Replacement Using In Vitro Cell Cultures:

- Researchers established an in vitro thermal pretreatment and injury challenge model to allow for the rapid evaluation of pharmacologic agents against thermal injury. This model generated gene-based therapeutic hypotheses that, if successful, may be transitioned to small animal models.
- A small animal model that displays gastroenteritis and/or signs of the hemolytic uremic syndrome due to Shiga toxin-producing *E. coli* is urgently needed to design and test therapies. Researchers were commencing the development of a three-dimensional human intestinal organoid system that could be used in certain experiments as an alternative to the current mouse model.
- Researchers worked on developing a three-dimensional human bladder organoid system that could be used in certain experiments as an alternative to the current mouse model.

Nonmammalian Species or Species Lower on the Phylogenetic Scale:

- Pharmacological studies employing visually stimulating testing and cognitively challenging behavioral tests are being conducted in mice and rats instead of macaques.
- A pilot study was conducted to discover and clone genes that can serve as toxicity markers in the Japanese medaka fish.

Reduction

Substitution of Computer Simulation, Models, or Other Technologies:

- In vaccine development, the identification of immunological target epitopes by computer searching eliminates the trial and error of finding appropriate targets, thereby reducing the number of experiments that fail.
- The use of membrane blood feeding is being further developed as an alternative to mice to maintain mosquito and mite colonies as a source of biological material to conduct studies on the transmission of malaria, dengue, and scrub typhus.
- Investigation of gene expression profiles in cell cultures exposed to toxic chemicals is anticipated to enhance in vitro toxicity testing and reduce the numbers of animals needed.
- The incorporation of telemetry into a more traditional toxicity protection study will ultimately reduce the number of animals necessary for these and future studies while increasing the amount and quality of information obtained per animal. Rat cadavers from rodent handling classes were used to practice probe placement before surgery was performed on live animals.

Utilization of Alternative Biological Testing Methods:

- Preliminary studies using research-grade plasmids have reduced the number of animals required to test vaccine lots.
- Boosting the immune system of mice with stimulants is being developed to yield wider ranges and higher titers of antibodies. This would result in the use of fewer mice than if antigen was used alone.

Refinement

Environmental Enrichment:

- Environmental enrichment is being developed for NHPs by engaging them in behavioral interaction that emulates the essential features of natural foraging. The results will be used to further refine the environmental condition of captive NHPs and ensure their psychological well-being.
- Novel strategies and methods for improved environmental enrichment are being evaluated for many different animals.
- Testing and validation of an alternative, rapid method for taking intraocular pressure measurements requiring no manual animal restraint, use of anesthetics, or sedation.

Reduce Pain and Distress:

- Studies were conducted to develop methods with which to collect free-catch urine samples and avoid the invasive procedure of cystocentesis for urine collection.
- Dogs used in the training of canine anesthesia were also used in the instruction of care, handling, and management classes.
- Efforts are under way to identify alternate markers for the successful development of an immune response.

3.5 SUMMARY

Each year, new techniques and capabilities improve the handling, treatment, and use of animals in RDT&E and training and potentially reduce the need for animals in those same endeavors. In FY02–FY03, there was significant evidence of the DoD’s aggressive pursuit to develop alternatives to replace, reduce, and refine the use of animals (examples are highlighted in Section 3.4). In addition to these developmental efforts, animal use data for FY02–FY03 indicate the widespread implementation of validated alternatives. Fish and frogs are replacing the use of many mice and rats while rats and mice continue to replace NHPs and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. The number of large animals used by the DoD over

the past decade has been significantly reduced, and some large species are rarely used at all. The use of sophisticated computer simulators in advanced trauma and life support training has reduced or completely eliminated large animals such as sheep in some institutions. While FY03 showed an increase in NHP use, the use of dogs and cats has decreased by 97% and 73%, respectively, relative to FY94. Together, three groups still represent less than 1% of the total animals used in research by the DoD. These and other examples of the development and implementation of new alternatives have translated into reductions in the overall use of animals higher on the phylogenetic scale. The animal use alternatives under reduction, replacement, and refinement constitute key initiatives in the biomedical RDT&E and educational training programs of the DoD.

The third section of this report can only partially document the persistent, ongoing efforts of DoD institutions to implement internal policies driving the refinement, reduction, and replacement of animals used in training and laboratory research. Just as the DoD exceeds AWA reporting requirements in accounting for animal use, this Department exceeds external, federal regulations and policies governing the humane treatment of animals. The DoD mandates its animal use oversight bodies to review each protocol under consideration to ensure the implementation of the most favorable animal use alternatives in both animal maintenance and research. However, this spirit is carried even further with DoD-wide initiatives that are clearly demonstrated by commitments to scientific research, initiatives, and conferences specifically targeted at developing and implementing new animal use alternatives in refinement, reduction, and replacement.

APPENDIX A

DoD DIRECTIVE ON ANIMAL USE



Department of Defense DIRECTIVE

NUMBER 3216.1

April 17, 1995

Certified Current as of December 1, 2003

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, and Section 2241 of title 10, United States Code
 - (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

1. REISSUANCE AND PURPOSE

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

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

2. 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 the "DoD Components") that perform or sponsor activities using animals.

3. DEFINITIONS

Terms used in this Directive are defined in enclosure 2.

4. DoD POLICY

4.1. 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.

4.2. 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).

4.3. 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).

4.4. 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.

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

4.6. 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)).

4.7. 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 4.8. 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.

4.8. 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).

4.9. A headquarters-level administrative review shall be conducted for proposals involving the use of nonhuman 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.

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

4.11. 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.

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

4.12.1. 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.

4.12.2. 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.

4.12.3. 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 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.

4.13. 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.

4.14. 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.

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

4.16. Personnel with complaints of violation of this Directive shall report such violations to either of the following members of the organization or facility: The IACUC chairperson, the attending veterinarian, the facility Commander, or the 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.

5. RESPONSIBILITIES

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

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

5.1.2. 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.

5.1.3. 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.

5.2. The Heads of the DoD Components shall:

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

5.2.2. 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.

5.2.3. Provide members to JTWG, as required.

5.2.4. Designate the appropriate office(s) within the DoD Component that shall perform the headquarters-level administrative review of proposals requiring the use of nonhuman primates and shall serve as the office where exemptions under paragraph 4.2., above, may be approved.

5.2.5. 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.

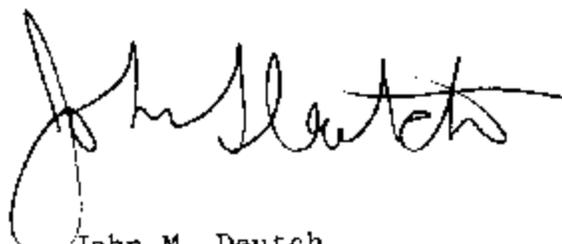
5.3. The Secretary of the Army shall:

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

5.3.2. 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.

6. EFFECTIVE DATE

This Directive is effective immediately.



John M. Deutch
Deputy Secretary of Defense

Enclosures - 3

- E1. References, continued
- E2. Definitions
- E3. Guidance Documents

E1. ENCLOSURE 1

REFERENCES, continued

- (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) Section 3109 of title 5, United States Code

E2. ENCLOSURE 2

DEFINITIONS

E2.1.1. 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.

E2.1.2. 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.

E2.1.3. 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.

E2.1.4. Research, Development, Test, and Evaluation. All activities that 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.

E2.1.5. 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.

E2.1.6. 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).

E3. ENCLOSURE 3

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

E3.1.1. The following documents provide national standards and guidance for the protection, treatment and use of animals:

E3.1.1.1. Animal Welfare Act (Sections 2131-2158 of title 7, United States Code, 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.

E3.1.1.2. Endangered Species Act of 1973 (Sections 1531-1543 of title 16, United States Code, 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.

E3.1.1.3. Marine Mammal Protection Act (Sections 1361-1384 of title 16, United States Code, 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.

E3.1.1.4. 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.

E3.1.1.5. Lacey Act (Section 42 of title 18, United States Code, as amended, and Title 50, Code of Federal Regulations, Part 16 and Subpart B, implementing rules and regulations). A program under the U.S. Fish and Wildlife Service, Department of the Interior. Prohibits the importation of certain wild animals or their eggs if the Secretary of the Interior determines that they are injurious to humans, the interest of agriculture, or other specified national interests.

E3.1.1.6. 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.

E3.1.1.7. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 309 West Clark Street, Champaign, IL 61820, March 1988. Provides guidelines for the care and use of the major agricultural animal species in the United States in research and teaching.

APPENDIX B

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



OFFICE OF THE SECRETARY OF DEFENSE

WASHINGTON, D.C. 20301

10 APR 1995

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

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

References:

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

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

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

Definition:

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

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

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

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

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

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

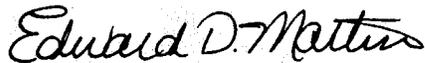
The following recommendations from the DoD Inspector General have been adopted as policy and shall be fully implemented by DoD Components which use animals in DoD-sponsored programs.

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

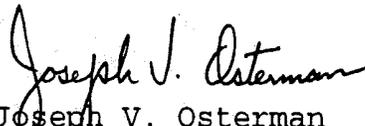
b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for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

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

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



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



Joseph V. Osterman
Director, Environmental
and Life Sciences

Attachments:

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

APPENDIX C

DoD STANDARD IACUC PROTOCOL FORMAT INSTRUCTIONS

Appendix C DOD Animal Use Protocol Format

C-1. Requirements

All DOD animal use protocols must use the format shown in this appendix. This protocol format includes requirements of the Animal Welfare Act Regulations, the Guide, and other applicable Federal regulations and DOD directives.

C-2. Protocol cover sheet

Before the protocol is submitted for IACUC review, at least three signatures are required on the protocol cover sheet (fig C-1). They must include those of the Principal Investigator (P.I.); either the department or division chief or the scientific review committee chairperson; and the individual performing the statistical review.

-
- I. Name of Facility
 - II. Proposal Number
 - III. Title
 - IV. Principal Investigator(s)/Division/Phone/E-mail
 - a. Printed Name (First Name, MI, Last Name); Title; Division
 - b. Signature; Date (YYYYMMDD); Phone, Fax
 - V. Scientific/Division Review/Phone/E-mail
 - a. Printed Name (First Name, MI, Last Name); Title; Division
 - b. Signature; Date (YYYYMMDD); Phone, Fax
 - VI. Statistical Review/Division/Phone/E-mail
 - a. Printed Name (First Name, MI, Last Name); Title; Division
 - b. Signature; Date (YYYYMMDD); Phone, Fax
 - VII. Attending Veterinarian/Division/Phone/E-mail
 - a. Printed Name (First Name, MI, Last Name); Title; Division
 - b. Signature; Date (YYYYMMDD); Phone, Fax

Figure C-1. DOD animal use protocol cover sheet

a. Scientific/division review. This signature verifies that the animal use proposal received appropriate scientific peer review and is consistent with good scientific practice.

b. Attending veterinarian. The Animal Welfare Act Regulations require that an attending veterinarian must be consulted in the planning of procedures/manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

c. Statistical review. A person knowledgeable in biostatistics is required to review all proposals to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

C-3. DOD animal use protocol format

a. The format shown in figure C-2 is designed to be used with several word-processing programs on a personal computer as a “fill-in-the-blank” type of document. It is available electronically through the appropriate DOD component oversight office listed in appendix B. Each paragraph and subparagraph in the format must have a response. Title headings do not require a response. Portions of the protocol format that are not applicable will be marked “N/A.” There are no space limitations for the responses. Pertinent standing operating procedures or similar documents that are readily available to the IACUC may be referenced to assist in the description of specific procedures.

PROTOCOL TITLE
PRINCIPAL INVESTIGATOR(S)
CO-INVESTIGATOR(S)
I. NON-TECHNICAL SYNOPSIS
II. BACKGROUND
II.1. Background
II.2. Literature Search for Duplication
II.2.1. Literature Source(s) Searched
II.2.2. Date of Search
II.2.3. Period of Search
II.2.4. Key Words of Search
II.2.5. Results of Search
III. OBJECTIVE/HYPOTHESIS
IV. MILITARY RELEVANCE
V. MATERIALS AND METHODS
V.1. Experimental Design and General Procedures
V.1.1. Experiment 1
V.1.2. Experiment 2
V.2. Data Analysis
V.3. Laboratory Animals Required and Justification
V.3.1. Non-animal Alternatives Considered
V.3.2. Animal Model and Species Justification
V.3.3. Laboratory Animals
V.3.3.1. Genus and Species
V.3.3.2. Strain/Stock
V.3.3.3. Source/Vendor
V.3.3.4. Age
V.3.3.5. Weight
V.3.3.6. Sex
V.3.3.7. Special Considerations
V.3.4. Number of Animals Required (By Species)
V.3.5. Refinement, Reduction, Replacement
V.3.5.1. Refinement
V.3.5.2. Reduction
V.3.5.3. Replacement
V.4. Technical Methods
V.4.1. Pain/Distress Assessment
V.4.1.1. APHIS Form 7023 Information (See attending veterinarian for assistance)
V.4.1.1.1. Number of animals
V.4.1.1.1.1. Column C: ___(Animal #)
V.4.1.1.1.2. Column D: ___(Animal #)
V.4.1.1.1.3. Column E: ___(Animal #)
V.4.1.2. Pain Relief/Prevention
V.4.1.2.1. Anesthesia/Analgesia/Tranquilization
V.4.1.2.2. Pre- and Post-procedural Provisions
V.4.1.2.3. Paralytics
V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures
V.4.1.3.1. Sources Searched
V.4.1.3.2. Date of Search
V.4.1.3.3. Period of Search
V.4.1.3.4. Key Words of Search
V.4.1.3.5. Results of Search
V.4.1.4. Unalleviated Painful/Distressful Procedure Justification
V.4.2. Prolonged Restraint
V.4.3. Surgery

Figure C-2. DOD animal use protocol format

-
- V.4.3.1. Pre-surgical Provisions
 - V.4.3.2. Procedure
 - V.4.3.3. Post-surgical Provisions
 - V.4.3.4. Location
 - V.4.3.5. Surgeon
 - V.4.3.6. Multiple Major Survival Operative Procedures
 - V.4.3.6.1. Procedures
 - V.4.3.6.2. Scientific Justification
 - V.4.4. Animal Manipulations
 - V.4.4.1. Injections
 - V.4.4.2. Biosamples
 - V.4.4.3. Adjuvants
 - V.4.4.4. Monoclonal Antibody (MAbs) Production
 - V.4.4.5. Animal Identification
 - V.4.4.6. Behavioral Studies
 - V.4.4.7. Other Procedures
 - V.4.4.8. Tissue Sharing
 - V.4.5. Study Endpoint
 - V.4.6. Euthanasia

V.5. Veterinary Care

V.5.1. Husbandry Considerations

V.5.1.1. Study Room

V.5.1.2. Special Husbandry Provisions

V.5.1.3. Exceptions

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care

V.5.2.2. Emergency Veterinary Medical Care

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy

V.5.3.2. Enrichment Restriction

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING

VII. BIOHAZARD/SAFETY:

VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the P.I. unless directed by the IACUC.

IX. ASSURANCES: The law specifically requires several written assurances from the Principal Investigator. Please read and sign the assurances as indicated.

As the Principal 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 modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

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

Figure C-2. DOD animal use protocol format—Continued

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

F. Responsibility: I acknowledge the inherent moral, ethical 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," namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

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

H. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(PRINT) First Name, MI, Last Name of Principal Investigator

Signature

Date (YYYYMMDD)

Figure C-2. DOD animal use protocol format—Continued

b. Some information may be added to the format to meet local needs. However, all labeled paragraphs and subparagraphs will remain in the same relative order. The added information will be similar or complementary to the information requested. Other types of requirements specific to a given Service, command, or locale (such as budgeting information, local coordinating requirements, or specific scientific review requirements, and so forth) can be added by placing them in front or behind the standard format.

C-4. Protocol format with completion aids

The format shown in figure C-3 is the same protocol format as in figure C-2. Explanations have been added to aid in completing the protocol proposal.

PROTOCOL TITLE: Title must include species of animal(s) used in research.

PRINCIPAL INVESTIGATOR(S)
CO-INVESTIGATOR(S)

I. NON-TECHNICAL SYNOPSIS: Provide a brief, narrative description of the proposal that is easily understood by a high school graduate. Include animal use in your description. (NOTE: This information may be used to complete the DOD Annual Report to Congress.)

II. BACKGROUND

II.1. Background: Include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited, and a description of the general approach will be provided.

II.2. Literature Search for Duplication: This search must be performed to prevent unnecessary duplication of previous experiments. A search of the Biomedical Research Database (BRD) is required. In addition, a search of EITHER the Federal Research in Progress (FEDRIP) OR the Computer Retrieval of Information of Scientific Projects (CRISP) database is required. Requirements for additional searches are at the discretion of the IACUC.

II.2.1. Literature Source(s) Searched

II.2.2. Date of Search

II.2.3. Period of Search

II.2.4. Key Words of Search

II.2.5. Results of Search: Provide a narrative description of the results of the literature search.

III. OBJECTIVE/HYPOTHESIS: State the objective of this protocol or the hypothesis to be accepted or rejected. (NOTE: This information will be used to complete the DOD Annual Report to Congress.)

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

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This section includes an explanation of experimental design. Technical methodology need not be described in this section, rather, it should be described under paragraph V.4, Technical Methods. Provide a complete description of the proposed use of animals to include a summary table of the experimental groups. Succinctly outline the formal scientific plan and direction of experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design of each separate experiment in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. A clearly understandable description of the numbers of animals and their distribution into experimental groups is essential. The number requested must equal the minimum number required to complete the study yet be sufficient to yield meaningful results. The minimum number includes animals necessary for controls or technique development, and so forth. Inclusion of a summary table or flow chart showing the distribution of animals by experimental group is highly recommended. The total number of animals required for the study is listed in section V.3.4.

V.1.1. Experiment 1

Figure C-3. DOD animal use protocol format with completion aids

V.1.2. Experiment 2

V.2. Data Analysis: List the statistical test(s) planned or describe the strategy intended to evaluate the data. Describe the statistical methodology used to determine group size and total number of animals. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, estimated effect size and expected variability. Be certain to include animals necessary for controls or technique development, and so forth.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: State all non-animal alternatives (for example, computer modeling, in vitro cell culture work) that were considered. Explain why animals are needed.

V.3.2. Animal Model and Species Justification: Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model? If less sentient (invertebrate versus vertebrate) animal models were considered but not chosen, explain why.

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species

V.3.3.2. Strain/Stock: If inbred or specialized animals are required, use proper terminology. (See the attending veterinarian for assistance.)

V.3.3.3. Source/Vendor: Provide a preferred source for the animals. Animals will be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with Code of Federal Regulations, Title 9, Animals and Animal Products, Chapter 1, Subchapter A, Animal Welfare, Parts 1, 2, and 3. (See the attending veterinarian for assistance.)

V.3.3.4. Age

V.3.3.5. Weight

V.3.3.6. Sex

V.3.3.7. Special Considerations: List specialized requirements for animals here (for example, simian immunodeficiency virus or herpes antibody free, Pasteurella free, and so forth).

V.3.4. Number of Animals Required (By Species): The number of animals stated here must correspond exactly to that described in section V.1. If, during the completion of the protocol, additional animals are needed owing to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval of additional animals.

V.3.5. Refinement, Reduction, Replacement (3 Rs): Investigators are required to consider the 3 Rs when preparing an animal use research protocol. In the paragraphs below, describe all provisions in this protocol that refine, reduce, or replace the use of animals. Discuss what provisions were considered and why they were not chosen. If N/A is used, explain why.

V.3.5.1. Refinement: Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing refinements, list

Figure C-3. DOD animal use protocol format with completion aids—Continued

refinement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.2. Reduction: Procedures or measures taken to reduce the number of animals used. Examples of reduction include but are not limited to the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages. In addition to listing reductions that will be used, list reduction alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.3. Replacement: Procedures or measures that eliminate the use of animals. Examples of replacements include but are not limited to the use of non-animal models or less sentient animal species. In addition to listing replacements that will be used, also list replacement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.4. Technical Methods: This information must be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure, obtain a clear understanding of what is to be done and how the animals 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.

V.4.1. Pain/Distress Assessment: 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 may involve pain or distress, even if relieved by anesthetics or analgesics, the P.I. must consult with the attending veterinarian.

V.4.1.1. APHIS Form 7023 Information: (See your attending veterinarian for assistance.) The protocol must contain an estimate of the number of animals that will be counted in columns C, D, and E of the APHIS Form 7023, Annual Report of Research Facility. Columns C, D and E represent specific pain categories. (See below paragraphs, V.4.1.1.1.-3.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for one protocol to have animals listed in several columns. For instance, control animals may be placed in Column C while experimental animals may be placed in Column D, depending upon the nature of the protocol. Reflect use of more than one species of animals in a duplicate table. The total numbers reflected in these three columns will add up to the number of animals requested for the entire protocol in paragraph V.3.4.

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column C: __ (animal #)

Examples of research procedures/manipulations that would require an animal to be placed in Column C are studies involving not more than slight or momentary pain and/or distress in a human being to which that procedure is applied.

V.4.1.1.1.2. Column D: __ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column D are procedures where anesthesia or analgesia will be administered to avoid or effectively relieve pain or distress. General anesthesia given for surgical procedures, or the use of analgesia or anti-inflammatory agents are examples of this category.

V.4.1.1.1.3. Column E: __ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column E are procedures in which alleviation of pain or distress are contraindicated for a scientifically justifiable reason such as the experimental results are likely to be confounded if drugs relieving pain or distress were

Figure C-3. DOD animal use protocol format with completion aids—Continued

administered. Detailed justification for putting animals into this category is required below in paragraph V.4.1.4.

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to effectively relieve or prevent pain or distress if the study will cause more than slight or momentary pain or distress. If pain/distress relief/prevention is planned, specify agents to be used and when these agents will be given (pre-emptive or post-procedural). Provide agent, dosage, and frequency of administration.

V.4.1.2.2. Pre- and Post-procedural Provisions: Describe the provisions for both pre- and post-procedural care, including provisions for post-procedural observations and frequency of observations. (Information concerning pre- and post-surgical care should be listed in paragraphs V.4.3.1 and V.4.3.3). If analgesics are used for pain/distress relief, provide the frequency of administration, observational criteria utilized to determine if animals are experiencing pain or distress, and the location for the post-procedural care.

V.4.1.2.3. Paralytics: The use of paralytic agents without anesthesia is prohibited. Describe the monitoring method that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: Respond N/A if the animals will experience not more than momentary or slight pain or distress and are placed in column C of APHIS Form 7023. (See paragraph V.4.1.1.)

V.4.1.3.1. Source(s) Searched: Examples are AGRICOLA, MEDLINE, BIOSIS, Altweb, and so forth.

V.4.1.3.2. Date of Search

V.4.1.3.3. Period of Search

V.4.1.3.4. Key Words of Search: Examples are pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful experimental procedure, and so forth.

V.4.1.3.5. Results of Search: Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Act specifically states that the P.I. must provide a narrative description of the methods and sources, e.g., the Altweb (Johns Hopkins Center for Alternatives to Animal Testing), MEDLINE, Life Sciences Abstracts, AGRICOLA, and BIOSIS) that he/she used to determine that alternatives to the painful procedure were not available. Discuss alternatives (those that would meet your scientific objectives) considered but not chosen. The alternatives literature search **MUST** be performed even when animals are placed in Column D and the pain or distress is alleviated through the use of analgesics or anesthetics.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Procedures that cause more than slight or momentary pain or distress that is not alleviated through the effective use of anesthetics or analgesics must be justified on a scientific basis in writing by the P.I. This paragraph must be completed if there are ANY animals in this protocol that will experience unalleviated pain or distress.

V.4.2. Prolonged Restraint: Describe (period of restraint, method, and timing of animal observations, habituation/training of animal to restraint device) and justify in detail any prolonged restraint greater than 12 hours for nonhuman primates or in accordance with IACUC policy for other species. Examples of restraint methods are primate chairs, restraint boards, metabolism cages, and so forth. This section is not intended for short-term actions such as rabbit restraint for bleeding, and so forth.

V.4.3. Surgery: Major survival operative procedures on non-rodent species will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions.

Figure C-3. DOD animal use protocol format with completion aids—Continued

Non-survival operative procedures do not require a dedicated facility, but they should be performed using surgical gloves, mask, and clean instruments. Additionally, the surgical site should be clipped and cleaned prior to surgery. Major survival rodent surgery does not require a dedicated facility but it must be performed using aseptic technique; that is, aseptic patient preparation, surgical gloves, mask, and sterile instruments. A major operative procedure is defined as a procedure that penetrates and exposes a body cavity, or causes substantial or permanent impairment of physical or physiological function.

V.4.3.1. Pre-Surgical Provisions: Describe the provisions for pre-surgical care, including provisions for pre-surgical observations and frequency of pre-surgical observations. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the pre-surgical care.

V.4.3.2. Procedure: Describe in detail any surgical procedures planned.

V.4.3.3. Post-Surgical Provisions: Describe the provisions for post-surgical care, including provisions for post-surgical observations, frequency of post-surgical observations and criteria for early euthanasia owing to surgical complications or pain that cannot be relieved. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the post-surgical care.

V.4.3.4. Location: Give the location/room number for the proposed surgical procedure.

V.4.3.5. Surgeon

V.4.3.6. Multiple Major Survival Operative Procedures: The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures

V.4.3.6.2. Scientific Justification

V.4.4. Animal Manipulations: Describe any injections, sampling procedures, or other manipulations of the animals necessary for the study. A reference or SOP may be furnished to the IACUC to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections: Information must include route of injection, dosage, frequency, volume injected, needle size, and anatomic injection site.

V.4.4.2. Biosamples: Examples include cerebrospinal fluid taps, blood sampling, and biopsies. List volumes taken, sampling site, frequency of sampling, needle size, and method of sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

V.4.4.3. Adjuvants: List any adjuvants used and the plan for their use. Provide a scientific justification for the use of Complete Freund's Adjuvant (CFA) and discuss why other less reactive adjuvants cannot be used. Provide dosages, volumes, route, number of injection sites, and injection locations. Specify frequency and method of injection site monitoring and include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.4. Monoclonal Antibody (MAbs) Production: Provide a scientific justification for *in vivo* MAbs production. What *in vitro* methods of MAbs production were considered but not used? For *in vivo* MAbs production, specify the priming agent, animal monitoring frequency, number and frequency of abdominal taps, and fluid replacement therapy. Include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.5. Animal Identification: Describe the method of animal identification used in this study. Examples include microchips, tattoos, ear tags, and cage cards.

Figure C-3. DOD animal use protocol format with completion aids—Continued

V.4.4.6. Behavioral Studies: Fully describe the use of aversive stimuli, food or water restriction, and so forth, that would affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria (for example, weight loss or state of hydration) for temporary or permanent removal of the animal from the study. Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.4.7. Other Procedures: Describe all procedures which have not been explained in other sections of this proposal that will be performed while conducting this research. Examples include electrocardiograms, radiology, and aerosol exposure.

V.4.4.8. Tissue Sharing: List what tissues will be shared, with whom, and for what purpose.

V.4.5. Study Endpoint: State the projected study endpoint for the animals (for example, recovery and return to issue pool, euthanasia, or death without early euthanasia). Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P.I. must ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be expeditiously removed from the study. Define specific criteria that will be used to determine study endpoint (for example, weight loss, loss of locomotion and significant lowering of body temperature, decreased food or water consumption, and decreased activity). Specifically address and scientifically justify any proposal in which critically ill or moribund animals are allowed to die as a result of the experimental procedures without the benefits of veterinary medical treatment or early euthanasia. Explain the plan for the disposition of surviving animals or animals removed from the study prior to its completion.

V.4.6. Euthanasia: If applicable, discuss the euthanasia method. The Animal Welfare Act defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current American Veterinary Medical Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. If requested, the attending veterinarian will assist in selecting the best method for euthanasia.

V.5. Veterinary Care: If requested, the attending veterinarian of the facility will assist PIs with preparing this section.

V.5.1. Husbandry Considerations: Federal regulations require that animal housing and living conditions must be appropriate to their species and contribute to their health and comfort. Briefly describe animal husbandry to include routine animal observations, caging methods, feed and water provisions, environmental parameters, sanitation schedules, and light cycles.

V.5.1.1. Study Room: Where will the experimental procedure be conducted? Will the animal be housed in this room for more than 12 hours?

V.5.1.2. Special Husbandry Provisions: Examples include micro-isolators, metabolic cages, food and water restriction.

V.5.1.3. Exceptions: Describe any deviations/exceptions to *The Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act regulations, or IACUC policy that have an impact on animal housing space, feeding, and sanitation. Deviations/exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Describe the routine veterinary medical care. State if the animals will be observed daily or more frequently. Indicate what will happen if the animal becomes ill or

Figure C-3. DOD animal use protocol format with completion aids—Continued

debilitated during the study and requires evaluation. List the criteria used for health evaluation while the animals are on study (for example, weight loss, ruffled fur, dehydration, decreased activity, and hunched body position). Include a response plan (for example, alternative early endpoint and veterinary medical treatment) in the event of debilitating illness or an adverse reaction.

V.5.2.2. Emergency Veterinary Medical Care: Describe emergency veterinary medical care.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: Discuss enrichment provided to animal species listed in this protocol.

V.5.3.2. Enrichment Restriction: Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of nonhuman primates and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING: List the names, qualifications and training by procedure of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures, and/or manipulations described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Contact the attending veterinarian for assistance with this requirement.

VII. BIOHAZARD/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal (for example, viral agents, toxins, radioisotopes, oncogenic viruses, and chemical carcinogens). Describe safety precautions and programs designed to protect personnel from biohazards associated with this research and any surveillance procedures in place to monitor potential exposures.

VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the PI unless directed by the IACUC.

Figure C-3. DOD animal use protocol format with completion aids—Continued

C-5. Personnel qualifications.

a. A Study Personnel Qualifications/Training table must be included in section VI of the protocol description. The table format is preferred by the IACUC for ease of reviewing the protocol. The table will contain the following four column headings:

- (1) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a dog).
- (2) Name of the person performing the activity.
- (3) Qualifications of the person performing the activity (for example, assistant laboratory animal technician (ALAT), 2 years experience).
- (4) Training of the person performing the activity (for example, Canine Procedures Workshop, 1999).

b. Itemize each activity being performed in the protocol. List per species if there are multiple species in the protocol. If more than one individual is performing the activity, list each individual separately.

APPENDIX D

DoD SEMIANNUAL PROGRAM REVIEW AND FACILITY INSPECTION CHECKLIST

Appendix D

Instructions for Use of DD Form 2856 (DOD Semiannual Program Review/Facility Inspection Checklist)

D-1. The checklist and the inspection report

The IACUC must complete the DOD Semiannual Program Review/Facility Inspection Checklist during the IACUC semi-annual program review and facility inspection in accordance with Title 9, Code of Federal Regulations, Subchapter A, Part 2, Subpart C. Individual checklists must be kept on file in the IACUC office but do not require attachment to the finished IACUC Semiannual Program Review/Facility Inspection Report.

D-2. Use of the form

The use of the form is self-explanatory; simply place a checkmark in the most appropriate category for each item on the inspection list. A sample completed DD Form 2856 is shown in figure D-1.

DOD SEMIANNUAL PROGRAM REVIEW/ FACILITY INSPECTION CHECKLIST				
ORGANIZATION			DATE OF REVIEW (YYYYMMDD)	
DOD Animal Facility			2002 Jul 19	
Completion of this checklist by the IACUC during the semi-annual program review and facility inspection is mandatory. Mark X in the most appropriate category for each item. KEY: A = Acceptable; M = Minor deficiency; S = Significant deficiency (is or may be a threat to animal health or safety).				
CATEGORIES	A	M	S	N/A
SECTION I - INSTITUTIONAL POLICIES AND RESPONSIBILITIES				
1. MONITORING THE CARE AND USE OF ANIMALS				
a. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE	X			
b. ANIMAL CARE AND USE PROTOCOLS	X			
c. PHYSICAL RESTRAINT	X			
d. MULTIPLE MAJOR SURGICAL PROCEDURES	X			
e. FOOD OR FLUID RESTRICTION	X			
2. PERSONNEL QUALIFICATIONS AND TRAINING				
3. OCCUPATIONAL HEALTH AND SAFETY OF PERSONNEL				
a. HAZARD IDENTIFICATION AND RISK ASSESSMENT		X		
b. PERSONNEL TRAINING	X			
c. PERSONAL HYGIENE	X			
d. FACILITIES, PROCEDURES, AND MONITORING	X			
e. ANIMAL EXPERIMENTATION INVOLVING HAZARDS	X			
f. PERSONAL PROTECTION	X			
g. MEDICAL EVALUATION AND PREVENTIVE MEDICINE FOR PERSONNEL	X			
SECTION II - ANIMAL ENVIRONMENT, HOUSING, AND MANAGEMENT				
4. PHYSICAL ENVIRONMENT				
a. MICROENVIRONMENT AND MACROENVIRONMENT		X		
b. HOUSING	X			
c. SPACE RECOMMENDATIONS	X			
d. TEMPERATURE AND HUMIDITY	X			
e. VENTILATION	X			
f. ILLUMINATION		X		
g. NOISE	X			
5. BEHAVIORAL MANAGEMENT				
a. STRUCTURAL ENVIRONMENT	X			
b. SOCIAL ENVIRONMENT	X			
c. ACTIVITY	X			
6. HUSBANDRY				
a. FOOD	X			
b. WATER	X			
c. BEDDING	X			
d. SANITATION	X			
e. WASTE DISPOSAL	X			
f. PEST CONTROL	X			
g. EMERGENCY, WEEKEND, AND HOLIDAY CARE	X			
7. POPULATION MANAGEMENT				
a. IDENTIFICATION AND RECORDS	X			
b. GENETICS AND NOMENCLATURE	X			

DD FORM 2856, AUG 2002

Figure D-1. Sample completed DD Form 2856

CATEGORIES	A	M	S	N/A
SECTION III - VETERINARY MEDICAL CARE				
8. ANIMAL PROCUREMENT AND TRANSPORTATION	X			
9. PREVENTIVE MEDICINE				
a. QUARANTINE, STABILIZATION, AND SEPARATION	X			
b. SURVEILLANCE, DIAGNOSIS, TREATMENT, AND CONTROL OF DISEASE	X			
10. SURGERY	X			
11. PAIN, ANALGESIA, AND ANESTHESIA	X			
12. EUTHANASIA	X			
SECTION IV - PHYSICAL PLANT				
13. FUNCTIONAL AREAS	X			
14. CONSTRUCTION GUIDELINES				
a. CORRIDORS	X			
b. ANIMAL ROOM DOORS	X			
c. EXTERIOR WINDOWS	X			
d. FLOORS	X			
e. DRAINAGE	X			
f. WALLS	X			
g. CEILINGS	X			
h. HEATING, VENTILATION, AND AIR CONDITIONING (HVAC)	X			
i. POWER AND LIGHTING		X		
j. STORAGE AREAS	X			
k. NOISE CONTROL	X			
l. FACILITIES FOR SANITIZING MATERIALS	X			
15. FACILITIES FOR ASEPTIC SURGERY	X			
REMARKS				
<p>3a. Risk assessment documentation partially complete.</p> <p>4a. Room appears overcrowded/cluttered.</p> <p>4f. Light flickering in Room 3.</p> <p>14i. Light cover cracked in Room 15.</p>				

DD FORM 2856 (BACK), AUG 2002

Figure D-1. Sample completed DD Form 2856—Continued

D-3. Evaluation guidelines

The DOD Semiannual Program Review/Facility Inspection Checklist was created using the National Research Council's 1996 *The Guide for Care and Use of Laboratory Animals* (Guide) as a template. Refer to the corresponding section of the Guide for more information on evaluation guidelines.

APPENDIX E

U.S. GOVERNMENT PRINCIPLES FOR ANIMAL USE

U.S. Government Principles for Animal Use

Interagency Research Animal Committee's

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

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

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

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

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APPENDIX F

BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS

BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS

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

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

Clinical Medicine (C1)

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

Clinical Surgery (C2)

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

Infectious Diseases (M2)

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

Medical Chemical Defense (M3)

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

Medical Biological Defense (M4)

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

Human Systems Technology (M5)

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

Combat Casualty Care (M6)

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

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

Ionizing Radiation (M7)

- Identification of protection against and treatment of radiation injury

Other Medical RDT&E (M8)

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

Physical Protection (N1)

- Updating of the national and international laser safety standards

Other Non-Medical RDT&E (N4)

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

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

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