
**DEPARTMENT OF DEFENSE
ANIMAL CARE AND USE PROGRAMS:
FISCAL YEARS 2004–2005**

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SECTION 1

INTRODUCTION

The report on Department of Defense (DoD) Animal Care and Use Programs for fiscal years 2004–2005 (FY04–FY05) was conducted by the Office of the Director, Defense Research and Engineering. In addition to a general program overview, this report provides a summary of DoD animal use with respect to research, development, test, and evaluation (RDT&E) and 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 RDT&E and training 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 RDT&E and training to apply for Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accreditation. DoD veterinarians, researchers, and policy makers 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, among other actions, 1) training requirements for nonaffiliated DoD Institutional Animal Care and Use Committee (IACUC) members, 2) implements a [standard format for animal use protocols](#), 3) implements a [DoD Semi-Annual Program Review and Facility Inspection Checklist](#), and 4) implements a reporting requirement for all DoD–sponsored 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 the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training](#), 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, medical education, and training programs is critical to the sustained technological superiority in military operations for the 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 faced around the world. DoD researchers are committed to providing this support, 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 the Department's scientists, medical personnel, and instructors, 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 supported and evaluated by and subsequently 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. Warfighter 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 current operations in Afghanistan and Iraq have yielded outbreaks of respiratory and diarrheal diseases such as shigellosis and parasitic diseases such as leishmaniasis, which threaten the health and well-being of our troops. The DoD also has 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 members 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 members. This is often provided by field medical personnel in austere, harsh, and hostile environments 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 warfighter and the medical supplies, experience, and expertise this person has. Currently, no *in vitro* model has been validated to 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 already have 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 personnel and civilians. 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 service members 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 medical care possible. 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 also are subjected to veterinary oversight, and these animals are maintained in facilities accredited by AAALAC International.

The use of animals is also important in the DoD's nonmedical programs. These programs develop biological sensors, sonar, echolocation, biorobotics, aviation construction materials, and hearing and eye protection systems. There is also nonmedical research 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 eventually will reduce the risks that our service members encounter in their daily duties. In performing marine biology research to better understand military working marine mammals, the DoD funds unique research that increases understanding of these fauna. Marine mammals are studied 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 provides resources for RDT&E and training missions to sustain the operational capabilities of today's service members. 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, 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 throughout the world. For example, DoD programs indirectly or directly advance our knowledge of infectious diseases, trauma care and treatment, respiratory injuries, burns, and specific surgical procedures. The DoD's role in these areas is critical because these areas typically receive only modest funding support in civilian research programs. In addition to benefiting humans, marine animals benefit from DoD research contributing to a better understanding of the impact of noise from ships on marine ecosystems.

With the end of the Cold War, Congress directed the DoD to manage additional medical research directly benefiting the civilian population, such as research on 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. service members who must operate in a global setting. In FY04–FY05, these research programs were awarded patents as shown in Table 1-2. While the underlying requirement for disease research is to protect U.S. service members, 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 at research. Because DoD personnel must operate in a worldwide theater, the DoD has had a long-standing commitment to the development of countermeasures to diseases prevalent in developing countries (for example malaria, which kills more people than any other disease worldwide). DoD scientists also collaborate closely with the National Institutes of Health 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 for 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 used to monitor warfighter health, the development of blood substitutes and agents for hemorrhage control, and the prevention of shock. Clinical and preclinical trials under way are addressing the efficacy of vaccines and/or treatments for malaria, HIV infection, anthrax, Ebola, and plague.

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 professionals 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 of research results in more humane methods of performing research that is applied in many types of research settings.

The benefits of scientific research using animals and of developing alternatives to animal use are customarily shared in publications. In FY04–FY05, the DoD contributed to more than 950 publications in scientific journals, proceedings, technical reports, books, and book sections.

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

- Development and evaluation of vaccines and therapeutic interventions against worldwide pathogens such as malaria, HIV, West Nile virus, avian influenza, meningococcus, and diarrheal pathogens
- Development and evaluation of countermeasures against biological threat agents including anthrax, typhus, shigella, brucellosis, and botulinum
- Research on hantavirus prevalence and transmission
- Research on enteric bacterial infections and parasitic flatworms
- Development of intravenous antimalarial drugs
- Epidemiologic surveillance of diseases endemic to tropical regions
- Development of new and effective drugs against chemical warfare agent exposure
- Development of advanced therapeutics for seizure prevention
- Research on traumatic brain injury resulting from hypoxia, hemorrhage, and hypercarbia
- Research on wound healing and antimicrobial interventions
- Development of diagnostic devices for combat medics
- Research for the treatment of traumatic brain injury
- Research on treatment and prevention of hemorrhagic shock
- Research on how to prevent acute and long-term neural damage after brain injury
- Evaluation of strategies for the radiotherapy of prostate cancer
- Evaluation of precision retinal laser therapy
- Quantification of munitions toxicity threats to wildlife
- Research on the causes of scarring following spinal cord injury
- Development of treatment against the effects of combat stress on brain function
- Development of methods to screen potential drug treatments for combat stress disorder
- Investigating the use of lithium in the treatment of bipolar disorder

Clinical Investigations

- Development of treatments for hypertension
- Development of new treatments and diagnostics for brown recluse spider bites
- Development of new screening methods for substance abuse
- Development of a model to identify a superior product for hernia repair and wound repair
- Research to further enhance the medical care and management of trauma patients
- Treatment and repair of upper intestinal injuries
- Development of an improved system for the treatment of bone infections (osteomyelitis)
- Development of improved methods for performing biopsies and removing kidney tumors
- Development of drugs to enhance wound healing
- Development of treatments that promote the healing of bone fractures

Training/Instructional

- General medical training of physicians
- Training of surgical residents in a variety of critical skills
- Advanced trauma life support and training in emergency medicine
- Training in emergency veterinary skills
- Training research and animal care personnel to improve veterinary handling techniques and procedures

Nonmedical RDT&E

- Developing methods and technologies for toxicity testing
- Evaluating the effects of human activity and military operations on marine mammals
- Testing effectiveness of ballistic body armor
- Development of a computer model capable of predicting blast effects and survivability
- Characterizing the neurophysiology of the hindbrain in support of developing therapeutic strategies for spinal cord and head injury
- Characterizing cellular and tissue profiles of the expression of genes, proteins, and protein pathways in the context of normal and injured tissues
- Identification of environmental and human health risk factors
- Developing detection and preventive measures for environmental toxins
- Evaluating toxicological hazards of occupational chemical exposure to heavy metals and other toxicants

Table 1-2 Patents Resulting from Animal Use Research in FY04–FY05

- Candidate vaccines for ebola virus
- A microsphere delivery system for the controlled release of anti-inflammatory drugs
- Vaccine candidates for malaria immunization
- A vaccine against the eastern equine encephalitis virus
- A field-based method for ecological risk assessment
- A vaccine and formulations for transcutaneous immunization
- Vaccine candidates against bacterial 'superantigens' such as those causing toxic shock syndrome
- A topical ointment for chemical blistering agents

Table 1-3 Examples of Humanitarian Benefits Resulting from DoD Research

The DoD works closely with the Centers for Disease Control and Prevention and the World Health Organization in managing worldwide health threats to both civilian and military populations. DoD surveillance and vaccine development efforts are critical to the worldwide monitoring of, and developing countermeasures and outbreak response plans to, potentially pandemic pathogens such as the avian influenza virus. In Central Asia, the DoD monitors the prevalence and spread of avian influenza in wild bird populations along major migratory routes, conducting thousands of cloacal swabs for analysis in capture-release programs. In Thailand, birds are being monitored for avian influenza virus to determine genetic variance among viral strains in support of vaccine design and development. The DoD also monitors antimicrobial drug resistance in both children with diarrheal diseases and DoD personnel serving overseas. These efforts have yielded critical information supporting preclinical immunogenicity studies using animals in vaccine development.

The health protection of service members stationed in developing nations places the DoD in a position to study serious diseases, such as malaria, that otherwise would not be a domestic priority in the United States. This has resulted in the development of effective vaccines for a number of pathogens prevalent in developing nations. The statistical power of serum samples from many thousands of service members places the DoD in a unique position of being able to conduct epidemiological studies that would be difficult to conduct in the public sector, which benefit both service members and civilians alike.

The DoD has engaged in numerous partnerships in the fight against malaria, working closely with the National Institute of Allergy and Infectious Diseases, commercial drug companies, and a variety of nonprofit foundations. 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 microbial resistance to current treatments. With some notable exceptions, civilian drug developers have shown reluctance to invest in malarial vaccines because of a low likelihood of profitability. 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.

Animals have been critical in supporting DoD efforts to develop effective, antigenic vaccines. A DoD-developed vaccine, not otherwise commercially available, is currently being used in East Africa to help stem an outbreak of Chikungunya virus. Previously, the DoD investigated the epidemiology of viral hemorrhagic and encephalitic diseases among civilians and deployed military troops in Peru, identifying arthropod-borne viruses commonly associated with human disease in the Amazon region such as dengue, Oropouche, and Venezuelan equine encephalitis (VEE). In addition, yellow fever, Mayaro, VEE, and one case by an apparently new Phlebovirus (family Bunyviridae) were isolated from febrile patients in an outbreak in the high jungle near Cusco, Peru. 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.

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 humans 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, tickborne encephalitis, hepatitis A, and *Staphylococcus* enterotoxins.

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 FY04–FY05. There are two major components of the FY04–FY05 report. Section 2 contains a summary of animal use with regard to DoD Components, species, research areas, and USDA pain categories. Section 3 describes the DoD initiatives to promote alternative methods that replace, reduce, or refine animal use. 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 organizations 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 FY04–FY05, data were acquired from 45 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 web site.

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, research areas, and USDA pain categories.

2.1 METHODS FOR DATA COLLECTION

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 FY04–FY05 as defined in Section 1.4.

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 and training. Only live animals that were used 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 program, work unit, or protocol. Breeding animals or animals on hand during FY04–FY05 but not actually used during the fiscal year are not included in the numbers reported here.

The DoD has a classification system for assigning all animals to one of five specific research areas. Within the 5 categories, there are a total of 20 subcategories (see Table 2-1). The five categories include: medical, clinical investigations, adjuncts/alternatives, training/instructional, and nonmedical. 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</p> <ul style="list-style-type: none"> M1: Military Dentistry M2: Infectious Diseases M3: Medical Chemical Defense M4: Medical Biological Defense M5: Military Operational Medicine M6: Combat Casualty Care M7: Ionizing Radiation M8: Other Medical RDT&E <p>CLINICAL INVESTIGATIONS (C)</p> <ul style="list-style-type: none"> C1: Clinical Medicine C2: Clinical Surgery C3: Other Clinical Investigations <p>ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A)</p> <ul style="list-style-type: none"> A1: Adjuncts to Animal Use Research A2: Alternatives to Animal Research A3: Other Alternatives/Adjuncts 	<p>TRAINING/INSTRUCTIONAL (T)</p> <ul style="list-style-type: none"> T1: Training, Education, and/or Instruction of personnel T2: Other Training/Instruction <p>NONMEDICAL (N) RDT&E</p> <ul style="list-style-type: none"> N1: Physical Protection N2: Physical Detection N3: Offensive Weapons Testing N4: Other Nonmedical RDT&E
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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 accordance with USDA guidance, 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) and therefore not reported to the USDA.

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

USDA COLUMN C

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

USDA COLUMN D

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

USDA COLUMN E

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

The animals assigned to Category C 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 assigned to Category D 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 assigned to Category E are those that experience, or may 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 Category 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 Pain Categories 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 Category 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. In addition to the veterinarian's review, the IACUC must review and approve all procedures before the study begins.

Since 1999, the total number of animals reported per year has been relatively steady, averaging at about 352,000 (Figure 2-1). Total annual use can show significant change over the years with the transient implementation of extramural research projects that employ large numbers of animals or with the conduct of intramural or extramural testing programs. The total values for FY04 and FY05 are 383,474 and 344,749, 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 27,653 and 33,702, respectively, comprising only 7.2% and 9.8% of the true total of animals

actually used in FY04 and FY05. Hence, the DoD’s nonrestrictive definition of *animal*, which includes all vertebrates from fish to NHPs, reflects a much higher level of accountability.

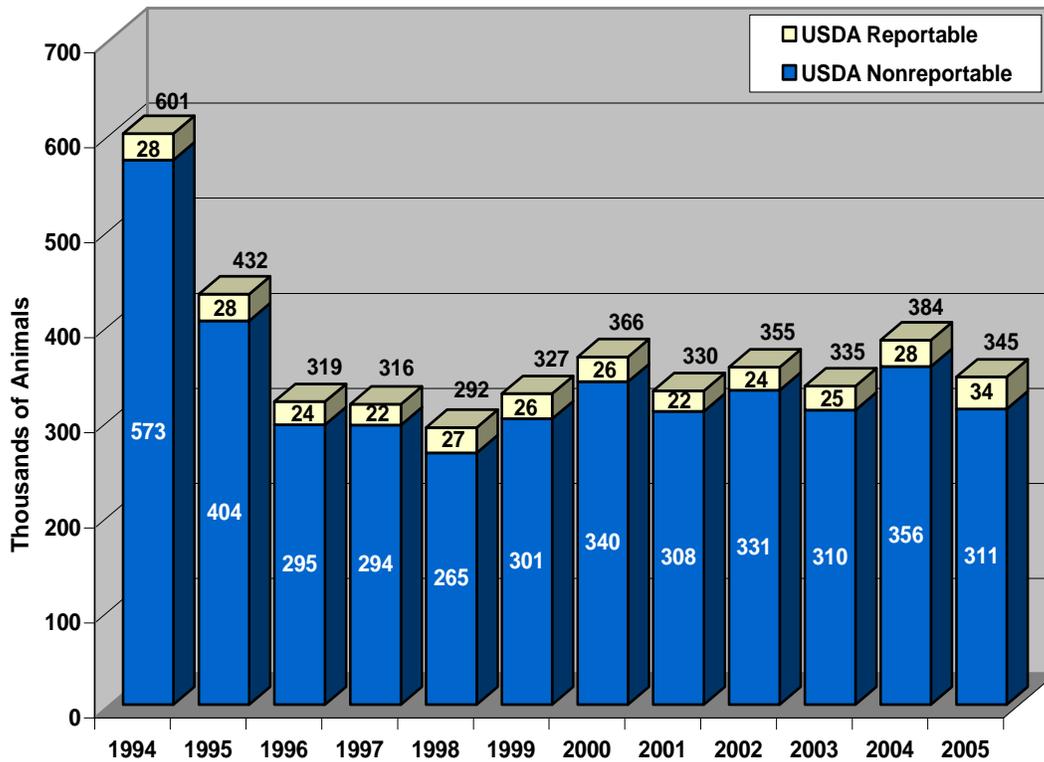


Figure 2-1 Animal Use by Fiscal Year (Totals above Columns)

In FY04 and FY05, 136,568 and 129,150 animals, respectively, were used in intramural research programs, and 246,906 and 215,599, respectively, were used in extramural grants or contracts (Figure 2-2). Both intramural and extramural numbers remain considerably lower than the FY94 peak usage values of 268,091 and 332,592, respectively. In FY05, intramural and extramural activities reflect respective declines of 52% and 35% 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*.

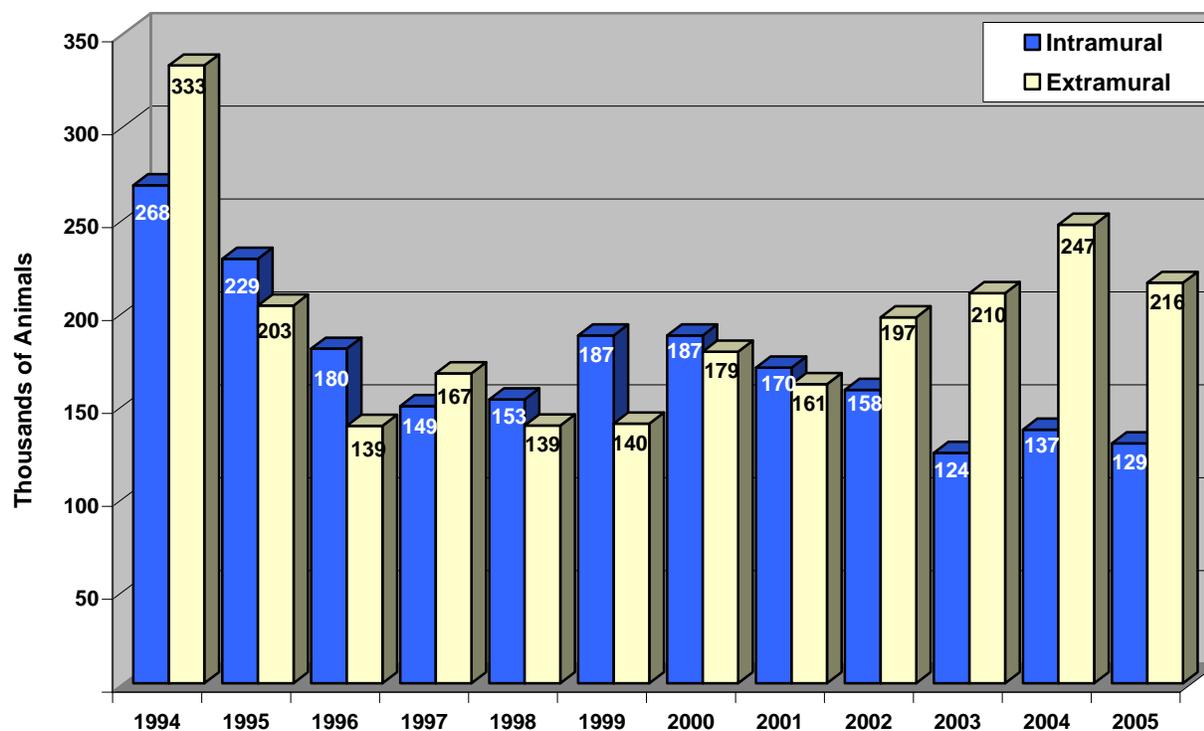


Figure 2-2 Intramural and Extramural Animal Use by Fiscal Year

Since FY94, there has been a remarkable decrease in animal use in both 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 largely funds non-DoD mission-required research that by FY05 comprised most of DoD animal use Category M8 (Other Medical RDT&E). Hence, 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, accounting for nearly 150,000 animals each year in FY04 and FY05 corresponding to about 40% of DoD animal use. When considering only DoD mission-required research, there has been a large decline in extramural animal use since FY94 when use exceeded 250,000 animals.

2.2 ANIMAL USE BY DOD COMPONENT

The total number of animals used by the DoD Components is presented in Figure 2-3. The DoD Components are grouped into four categories: Army, Navy, Air Force, and the remainder of the DoD Components. The last category, "Other DoD," includes but is not limited to the Uniformed Services University of the Health Sciences (USUHS), Defense Advanced Research Projects Agency, Armed Forces Radiobiology Research Institute, and Armed Forces Institute of Pathology. Figures 2-4 and 2-5 show the intramural and extramural animal use by DoD Components, respectively. FY03 data are included for comparison.

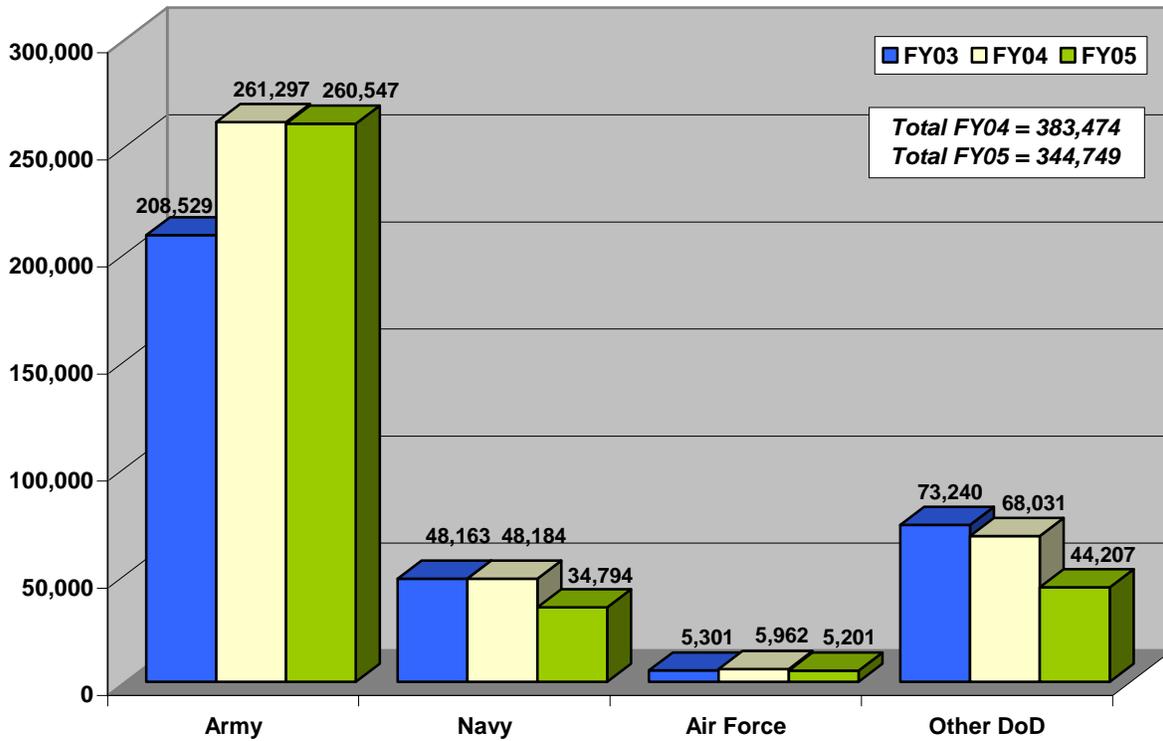


Figure 2-3 Animal Use by DoD Components for FY04–FY05 (FY03 for comparison)

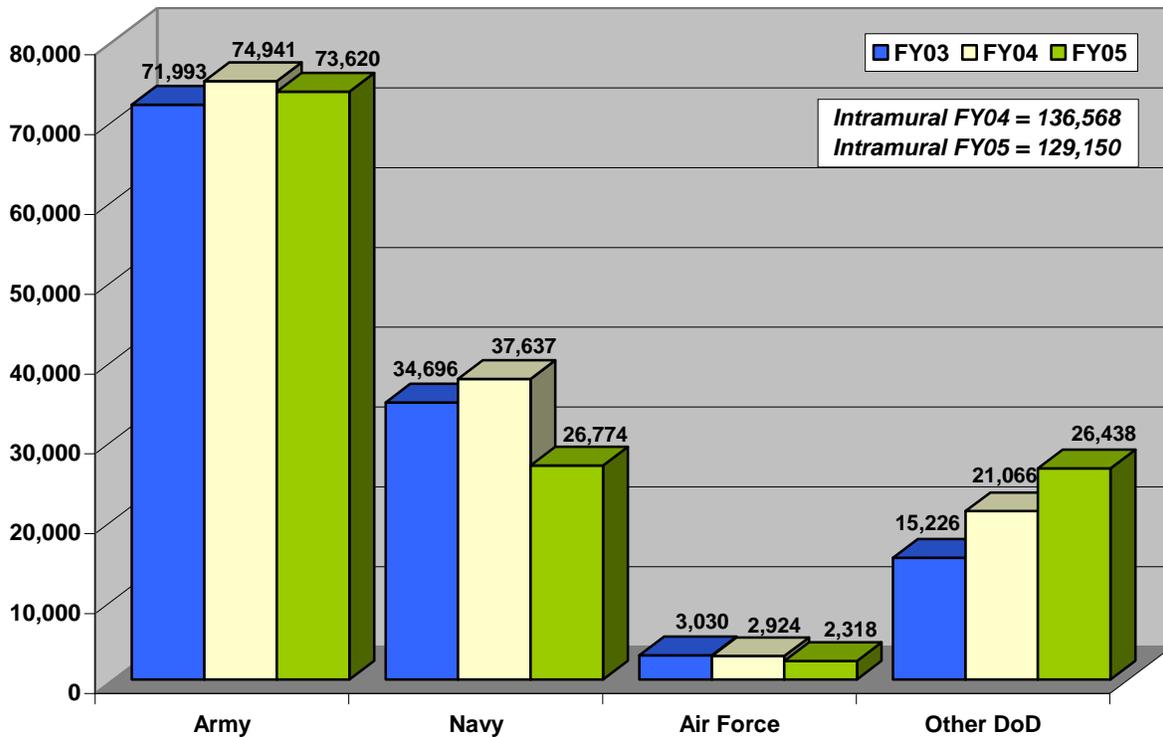


Figure 2-4 Intramural Animal Use by DoD Components for FY04–FY05 (FY03 for comparison)

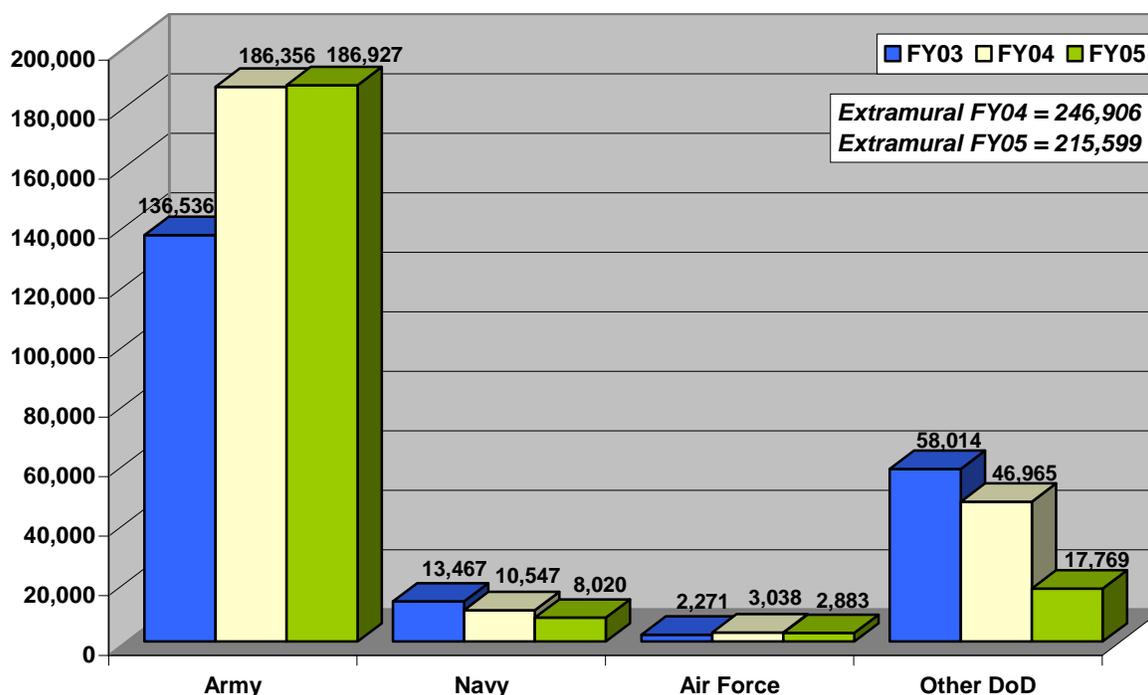


Figure 2-5 Extramural Animal Use by DoD Components for FY04–FY05 (FY03 for comparison)

Within the DoD, the Army is tasked with the greatest share of medical research conduct and oversight. The Army was responsible for 68% of the total number of animals used by the DoD in FY04, 55% of the total number of intramural animals, and 75% of the total number of extramural animals. Over the past 2 years, the Army's overall animal use has risen slightly, mainly attributable to extramural use. In FY05, the Army used 76% of all animals reported by the DoD with 59% being intramural and 87% extramural. Overall, however, the Army has decreased its use of animals in research by 57% since FY94. As pointed out in Section 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 68%. While Army programmatic funds have steadily risen, animal use has concurrently declined through the use of reduction initiatives.

Table 2-3 shows a list of CDMRP efforts with FY04 and FY05 funding, all of which are managed by the Army. Funding is dependent on yearly congressional appropriations. These programs used the majority (61%–69%) of the Army's extramural research animals and 39%–43% of the total DoD animal use in FY04–FY05. Among all of the Army's extramural programs, the Breast Cancer Research Program employed the largest number of animals. FY04 use (48,723) rose from FY03 (41,064) while levels declined again in FY05 (43,352). This specific program alone accounted for 20% of all animals used by the DoD for FY04–FY05.

Table 2-3 U.S. Army FY04–FY05 Congressionally Directed Medical Research Programs

Program	FY04	(\$ millions)	FY05
Alcoholism	4.5		3.8
Blood Cancers	8.5		4.3
Brain Tumors	0.0		4.9
Breast Cancer	152.0		153.0
Cancer Research, Various Areas	3.0		22.8
Chronic Myelogenous Leukemia	4.3		4.3
Lung Cancer	9.5		9.5
Muscular Diseases and Function	5.3		8.3
Neurofibromatosis	20.0		25.0
Orphan Disease Drug Discovery	0.0		2.0
Ovarian Cancer	10.0		10.0
Prion Research	1.5		0.0
Prostate Cancer	91.5		91.7
Peer Reviewed ¹	50.0		50.0
Tuberous Sclerosis	3.0		3.2
Other Medical Research	7.9		4.9

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 diseases and military dentistry research and is the DoD Executive Agency for medical chemical and biological defense and nutrition studies. In FY05, Army research on infectious diseases and chemical and biological defense used 34,748 and 43,701 animals, respectively. This was a 14% decrease for infectious disease research from FY04 and an 85% decrease from FY94.

The Navy used 13% of the total number of DoD animals in FY04 with this value declining to 10% in FY05. Since FY03, intramural animal use has remained steady while extramural use has seen some fluctuation. 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, both mosquito-borne diseases.

Unlike the Army, wherein most 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 (88% and 89% for FY04 and FY05, respectively) used by the Navy were used in medical research with 72% (34,543 for FY04) and 66% (22,923 for FY05) 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 4,867 and 3,960 animals in that area in FY04 and FY05 with 86% and 76% used in extramural activities, respectively.

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 FY04 and FY05, it used 1%–2% of the total number of animals reported used by the DoD. Over the past 3 years, Air Force intramural and extramural animal use has remained steady. The Air Force used 6% and 14% of animals in clinical investigation projects in FY04 and FY05, respectively, and 44% and 42% of its animals in nonmedical research studies.

The contribution of the Other DoD Components to the overall annual DoD total has continually decreased over the past 3 years. While intramural use by these DoD Components increased as compared to FY03 (16% in FY04 to 20% in FY05), extramural animal use decreased (19% in FY04 to 31% in FY05). Overall, these DoD Components used the majority of their animals (77% and 67% for FY04 and FY05, respectively) in medical research. Animal use in clinical research projects declined drastically from 13% in FY04 to 1% in FY05.

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). Total numbers of nonmammals showed some decline in FY04 (3,857) before increasing in FY05 (11,618) with the use of large numbers of fish and avian species in FY05. The other mammals category, which includes all nonrodent species, remained relatively constant at about 14,000 animals. The use of rodents increased in FY04 and then decreased to a more usual annual level in FY05. As in the previous section, FY03 values have been added to provide some continuity with the previous reporting period.

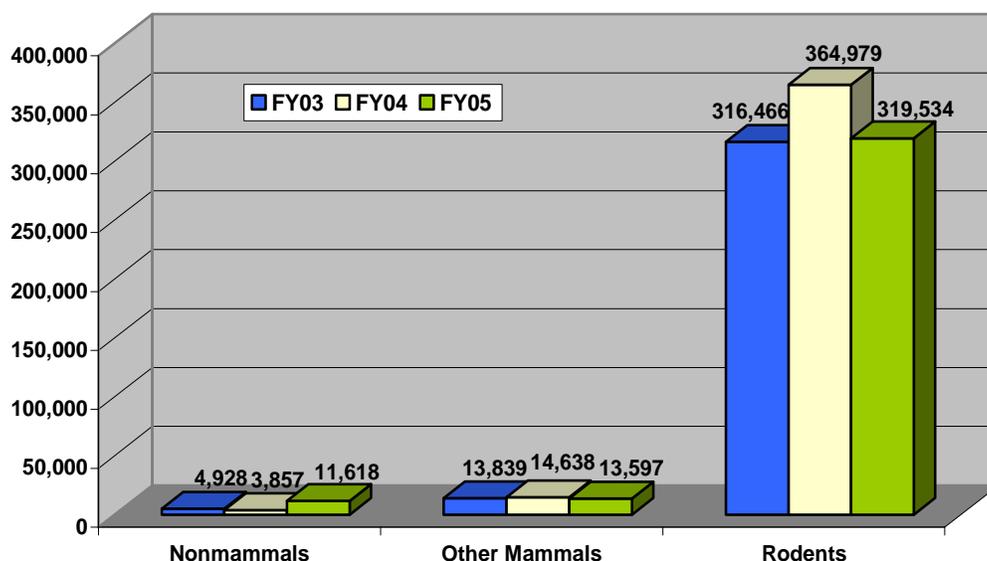


Figure 2-6 Nonmammals, Other Mammals, and Rodents for FY04–FY05 (FY03 for comparison)

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 FY04 and FY05, respectively.

Mice and rats, both classified as rodents, are considered phylogenetically the lowest mammalian species in preclinical research and accounted for 95% and 93% of the DoD's animal use in FY04 and FY05, 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 FY03 to FY05 period with rat use ranging from 36,714 (FY03) to 43,116 (FY04) and guinea pig use ranging from 6,307 (FY04) to 9,747 (FY05). The use of hamsters rose slightly from 4,098 in FY03 to 4,732 in FY05. There were also notable increases in the use of avian and fish species between FY04 and FY05 at 332% and 400%, respectively.

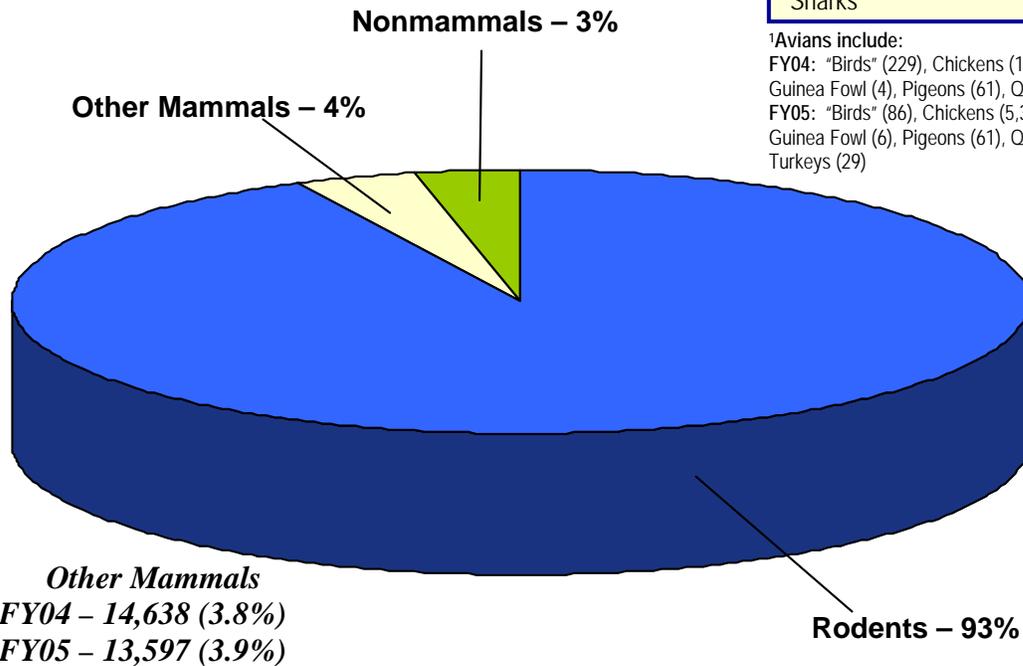
Total FY04 = 383,474
Total FY05 = 344,749

Nonmammals
FY04 – 3,857 (1.0%)
FY05 – 11,618 (3.4%)

Species	FY04	FY05
Amphibians	694	792
Avians ¹	1,829	6,079
Fish	1,030	4,117
Reptiles	209	575
Sharks	95	55

¹Avians include:
 FY04: "Birds" (229), Chickens (1,299), Geese (24), Guinea Fowl (4), Pigeons (61), Quail (188), Turkeys (24)
 FY05: "Birds" (86), Chickens (5,361), Ducks (384), Geese (25), Guinea Fowl (6), Pigeons (61), Quail (102), Sparrows (25), Turkeys (29)

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



Species	FY04	FY05
Bats	507	72
Black Bears	7	0
Cats	23	40
Cows/Bulls	10	6
Deer/Elk	15	100
Dogs	193	265
Ferrets	141	211
Goats	3,924	4,823
Hedgehogs	2	0
Horses	35	22
Marine Mammals ¹	105	26
Mongoose	2	1
Nonhuman Primates	1,962	1,891
Opossums	0	12
Pigs/Swine	3,678	2,735
Rabbits	3,566	2,907
Sheep	205	397
Shrews	223	62
Skunks	0	12
Voles	40	15

Rodents
FY04 – 364,979 (95.2%)
FY05 – 319,534 (92.7%)

Species	FY04	FY05
Chinchillas	187	70
Gerbils/Jirds	29	19
Guinea Pigs	6,307	9,747
Hamsters	4,531	4,732
Mice	310,586	265,725
Prairie Dogs	0	76
Rats	43,116	38,999
Squirrels	223	166

¹Marine Mammals include:
 FY04: Dolphins (16), Elephant Seals (31), Fur Seals (1), Sea Lions (18), Seals (6), Whales (33)
 FY05: Dolphins (16), Sea Lions (3), Seals (5), Whales (2)

Figure 2-7 Intramural and Extramural Animal Use by Species for FY04–FY05

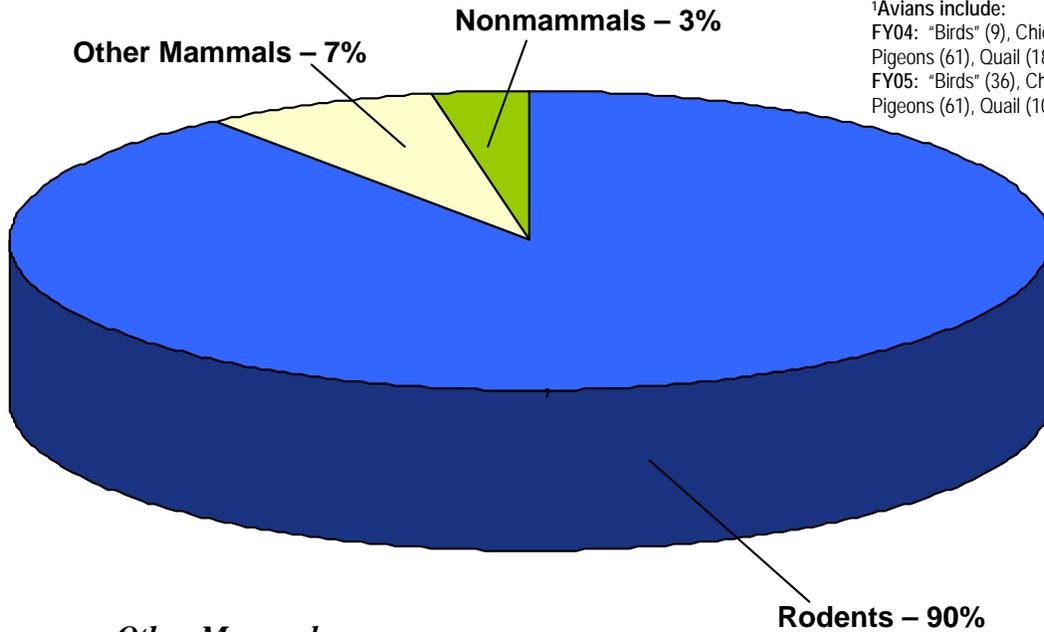
Intramural FY04 = 136,568
Intramural FY05 = 129,150

Nonmammals
FY04 – 2,437 (1.8%)
FY05 – 5,316 (4.1%)

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

Species	FY04	FY05
Amphibians	642	603
Avians ¹	951	2,445
Fish	669	1,994
Reptiles	175	274

¹Avians include:
 FY04: "Birds" (9), Chickens (669), Geese (24), Pigeons (61), Quail (188)
 FY05: "Birds" (36), Chickens (2,221), Geese (25), Pigeons (61), Quail (102)



Other Mammals
FY04 – 9,625 (7.0%)
FY05 – 10,189 (7.9%)

Species	FY04	FY05
Bats	491	0
Cats	23	40
Dogs	69	81
Ferrets	101	211
Goats	3,421	4,815
Gymnures	2	0
Horses	26	13
Marine Mammals ¹	15	11
Mongoose	2	1
Nonhuman Primates	1,379	1,541
Pigs/Swine	2,008	1,529
Opposum	0	12
Rabbits	1,701	1,621
Sheep	124	237
Shrews	223	62
Voies	40	15

¹Marine Mammals include:
 FY04: Dolphins (14), Whales (1)
 FY05: Dolphins (10), Whales (1)

Rodents
FY04 – 124,506 (91.2%)
FY05 – 113,645 (88.0%)

Species	FY04	FY05
Chinchillas	187	2
Gerbils/Jirds	29	19
Guinea Pigs	5,365	8,241
Hamsters	2,597	2,817
Mice	93,653	85,813
Rats	22,671	16,733
Squirrels	4	20

Figure 2-8 Intramural Animal Use by Species for FY04–FY05

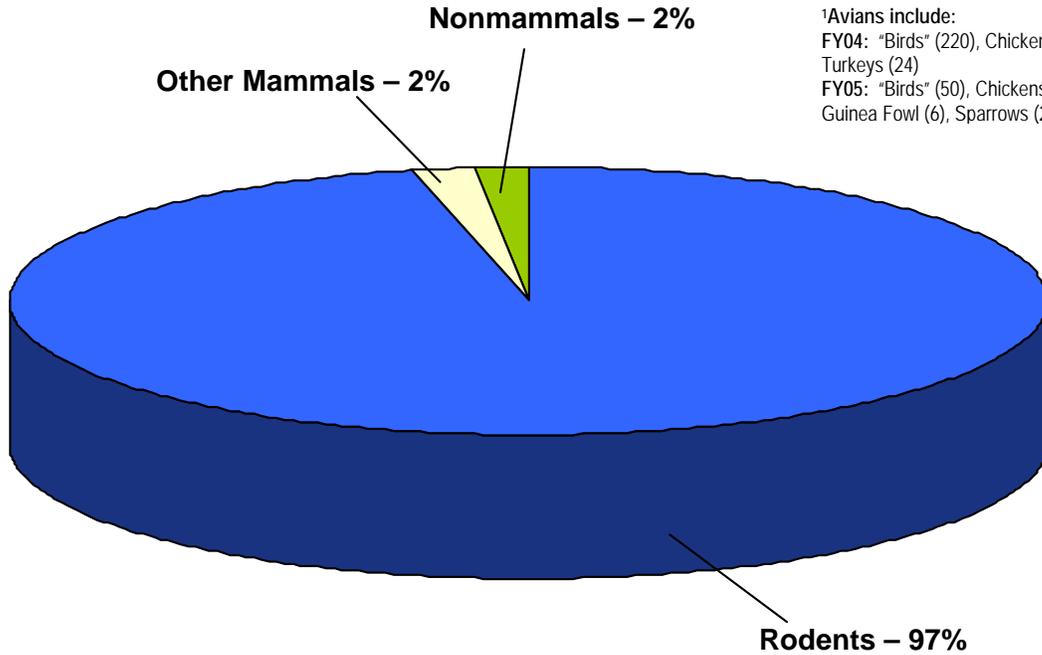
Extramural FY04 = 246,906
Extramural FY05 = 215,599

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

Nonmammals
FY04 – 1,420 (0.6%)
FY05 – 6,302 (2.9%)

Species	FY04	FY05
Amphibians	52	189
Avian ¹	878	3,634
Fish	361	2,123
Sharks	95	55
Reptiles	34	301

¹Avians include:
 FY04: "Birds" (220), Chickens (630), Guinea Fowl (4), Turkeys (24)
 FY05: "Birds" (50), Chickens (3,140), Ducks (384), Guinea Fowl (6), Sparrows (25), Turkeys (29)



Other Mammals
FY04 – 5,013 (2.0%)
FY05 – 3,408 (1.6%)

Species	FY04	FY05
Bats	16	72
Black Bears	7	0
Cows/Bull	10	6
Deer	15	100
Dogs	124	184
Ferrets	40	0
Goats	503	8
Horses	9	9
Marine Mammals ¹	90	15
Nonhuman Primates	583	350
Pigs/Swine	1,670	1,206
Rabbits	1,865	1,286
Sheep	81	160
Skunks	0	12

¹Marine Mammals include:
 FY04: Dolphins (2), Elephant Seals (31), Fur Seals (1), Sea Lions (18), Seals (6), Whales (32)
 FY05: Dolphins (6), Sea Lions (3), Seals (5), Whales (1)

Rodents
FY04 – 240,473 (97.4%)
FY05 – 205,889 (95.5%)

Species	FY04	FY05
Chinchillas	0	68
Guinea Pigs	942	1,506
Hamsters	1,934	1,915
Mice	216,933	179,912
Prairie dogs	0	76
Rats	20,445	22,266
Squirrels	219	146

Figure 2-9 Extramural Animal Use by Species for FY04–FY05

Figure 2-10 represents the use of NHPs, dogs, and cats between FY94 and FY05. The use of cats has remained relatively low since a small spike in FY00 with 23 in FY04 and 40 in FY05. Over the 2 years, all cats were used in intramural pediatric and veterinary training programs. The number of dogs used in research also declined in FY04 (193) and rose again in FY05 (265). This is still lower than that of the highest peak in the past 10 years (FY00). Over the FY04–FY05 period, 458 dogs were employed; the majority (49%) were used in nonmedical research, which includes physiology and neurobiology. Dogs are a unique animal model for both prostate cancer and leishmaniasis. Twenty-four percent of these were used in veterinary training. There was also an 18%–21% decline in the number of NHPs used in FY04 and FY05 relative to FY03. NHPs are unique in their ability to model human response to therapeutic compounds and are used in advanced preclinical research.

A cornerstone of the IACUC animal use review process is to ensure the use of the lowest possible animal species on the phylogenetic scale. Oversight of animal protocols requires a review to ensure the lowest possible species is being used. 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.

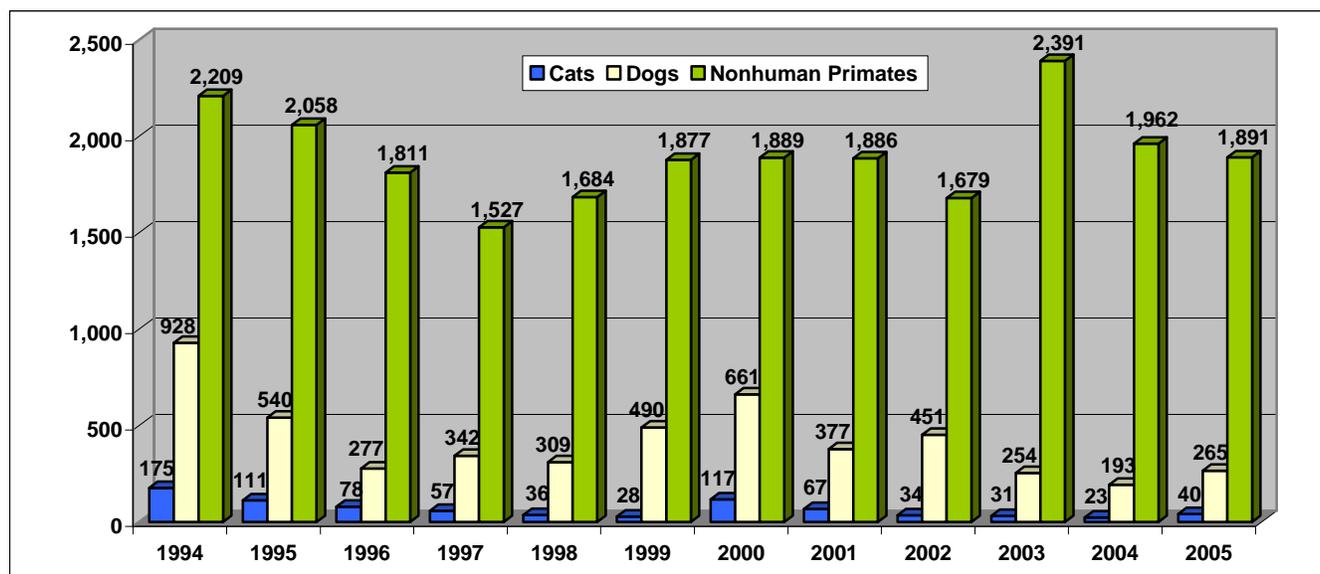


Figure 2-10 Use of Cats, Dogs, and NHPs by Fiscal Year

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 graphs are an enlargement of categories wherein animal use is dwarfed by that in the medical research category (M). FY03 data are included for comparison only.

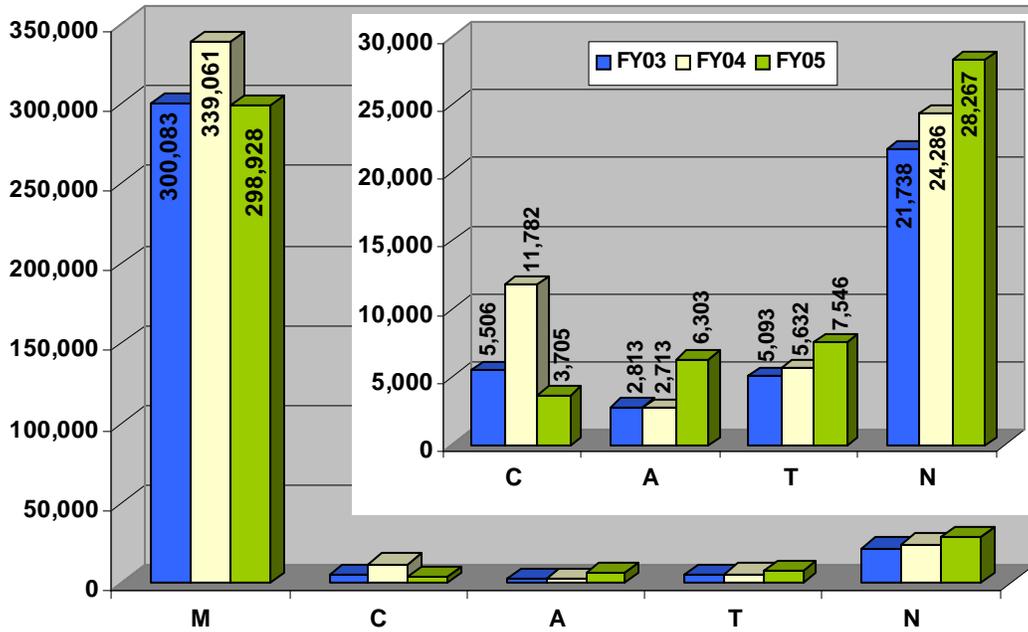


Figure 2-11 Animal Use by Animal Use Category for FY04–FY05 (FY03 for comparison)

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

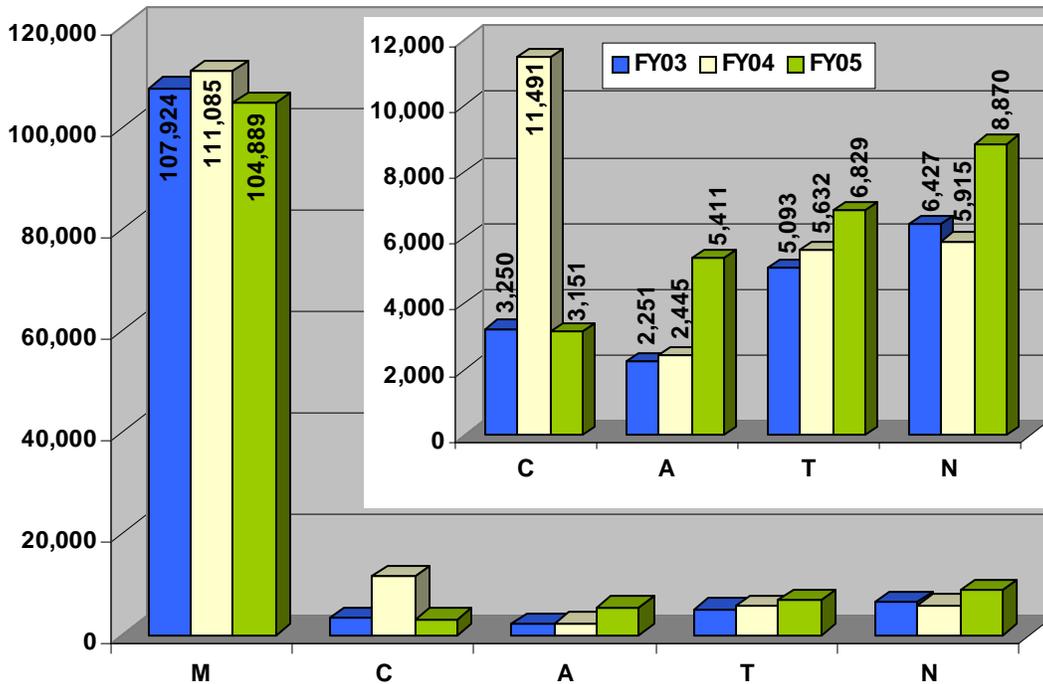


Figure 2-12 Intramural Animal Use by Animal Use Category for FY04–FY05 (FY03 for comparison)

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.

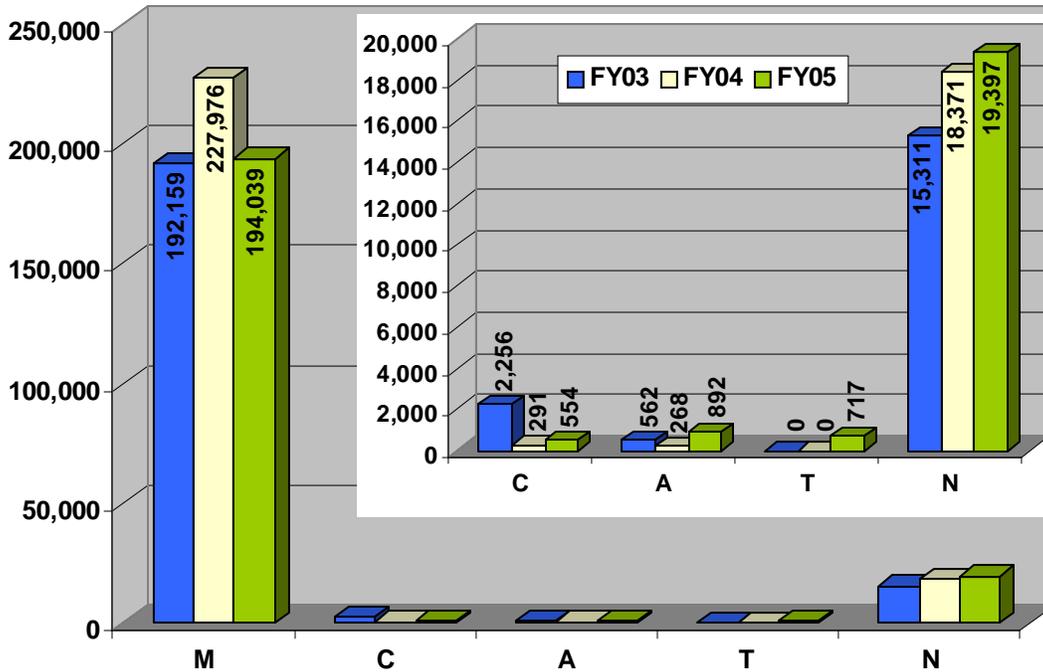


Figure 2-13 Extramural Animal Use by Animal Use Category for FY04–FY05 (FY03 for comparison)

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

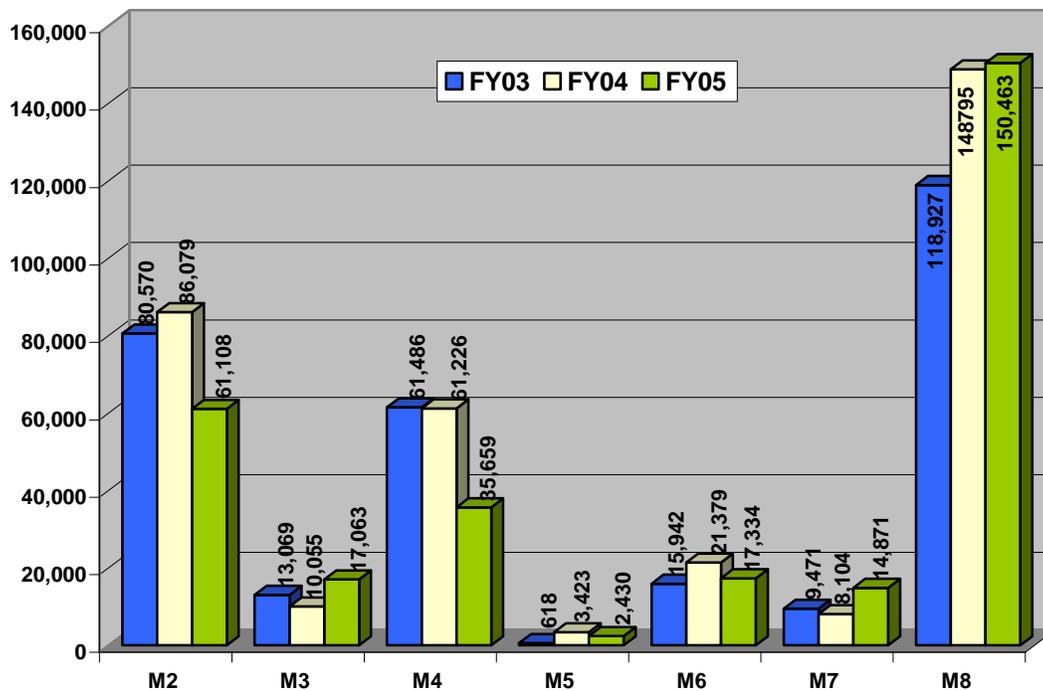


Figure 2-14 Animal Use by Subcategories of Medical Category RDT&E for FY04–FY05 (FY03 for comparison)

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).

Clinical Investigations (Category C) accounted for approximately 3% and 1% of the animals used by the DoD in FY04 and FY05, 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. This percentage drop is not likely to reflect any shift in priorities since those engaged in DoD animal use reporting may elect to categorize animal use in more defined categories such as M6 or the relatively specific subcategories of M8 (see Table 2-4).

Activities in the area of Adjuncts/Alternatives to Animal Studies (Category A) accounted for 2,713 and 6,303 animals in FY04 and FY05, respectively. This increase illustrates 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 FY04–FY05 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, researchers, and veterinary personnel.

Nonmedical RDT&E animal use (Category N) accounted for slightly more than 5% (24,286) and 8% (28,267) of the total FY04 and FY05 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. In FY04–FY05, nonmedical toxicity studies employed more than 3,000 fish, reflecting a deliberate effort to use the lowest species on the evolutionary scale. Over the 2 years, the majority of Category N animals (44%) were used in studies of physiology, especially in neurophysiology.

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, 87% of animal use by the DoD in FY04–FY05 was in the medical category (M). Figure 2-14 shows the breakout by medical subcategories. No animals were used in subcategory M1, military dentistry. There were several shifts in animal use levels among the different medical research category components between FY04 and FY05. Both infectious disease research (M2) and medical biological defense (M4) showed significant declines in animal use in FY05, at 29% and 42%, respectively, while medical chemical defense (M3) and ionizing radiation research (M7) showed significant (41%–46%) increases by FY05. In FY04 and FY05, 25% and 20%, respectively, of the animals used in medical RDT&E were in the area of infectious diseases (M2). The primary thrust of M2 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 FY04 and FY05, the medical chemical defense program (M3) used 3% and 6%, respectively, of all M category animals, and the medical biological defense program (M4) used 18% and 12% (61,226 and 35,659) 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 also has assumed a central role in homeland defense and the development of countermeasures to terrorist threats such as anthrax.

Animal use for the medical RDT&E subcategory M5, which addresses the bioeffects of laser exposure, blast overpressure, operational stress, and occupational health protection, increased from 618 in FY03 to 3,423 in FY04. Animal use in M6 research increased 25% between FY03 and FY04, reflecting studies 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 some change with animal use declining in FY04 (from FY03) before returning to greater levels in FY05.

M8 (Other Medical RDT&E) accounted for 44%–50% of the total medical category for FY04–FY05 (Figure 2-14). The CDMRP (Table 2-3) used approximately 150,000 animals per year in FY04 and FY05. These programs, which are managed by the Army, are primarily directed at cancer biology and account for nearly all M8 animals (Table 2-4), half of the animals used in the medical RDT&E category (M), and 39%–43% of the total

DoD animals used in FY04–FY05. 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 FY04 and FY05

Subcategory	Animals Used	
	FY04	FY05
Alcohol Research	4,079	2,971
Bone Health Research	19,935	5,572
Breast Cancer Research	48,723	43,352
Defense Women's Health	0	15
Disaster Relief Emergency Services	846	0
Environmental Safety	193	43
Gulf War Illnesses	704	537
Lung Cancer Research	8,446	655
Medical Laser Research	1,116	3,272
Neurofibromatosis Research	8,954	15,461
Neurotoxin Research	7,203	7,728
Other Targeted Disease Research	3,281	7,946
Ovarian Cancer Research	5,794	7,238
Prion Research	4,430	7,787
Prostate Cancer Research	34,521	44,346
oxicology	977	2,211
Undersea Research	536	949
Zoonosis	33	188
Total FY Values	149,771	150,271

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.

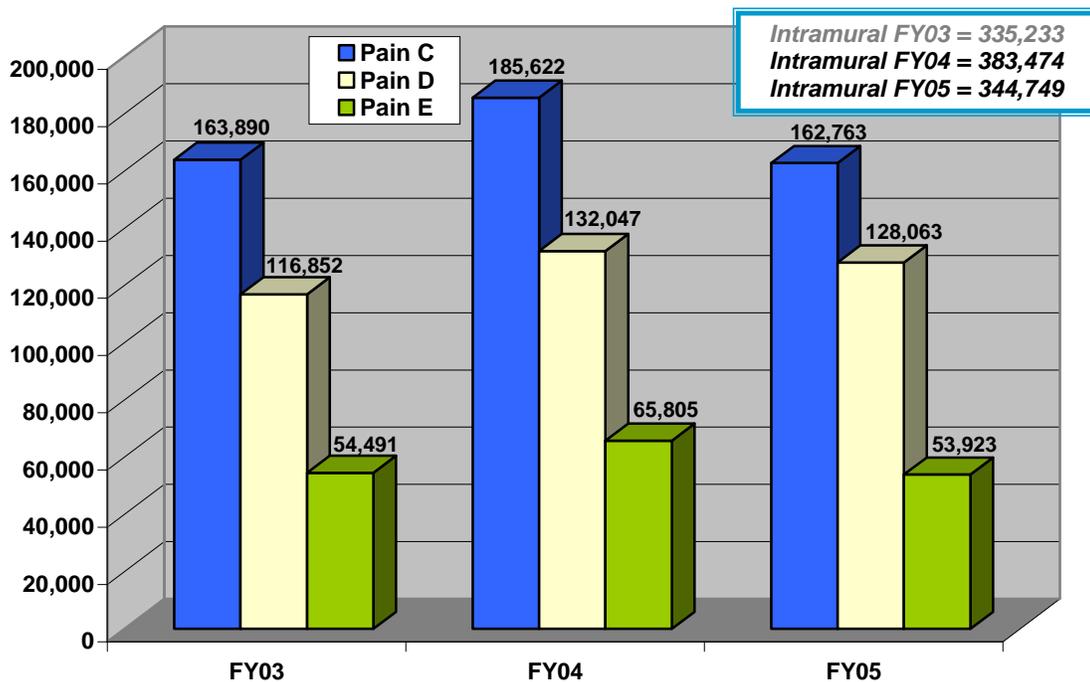


Figure 2-15 Animal Use by USDA Pain Category for FY04–FY05 (FY03 for comparison)

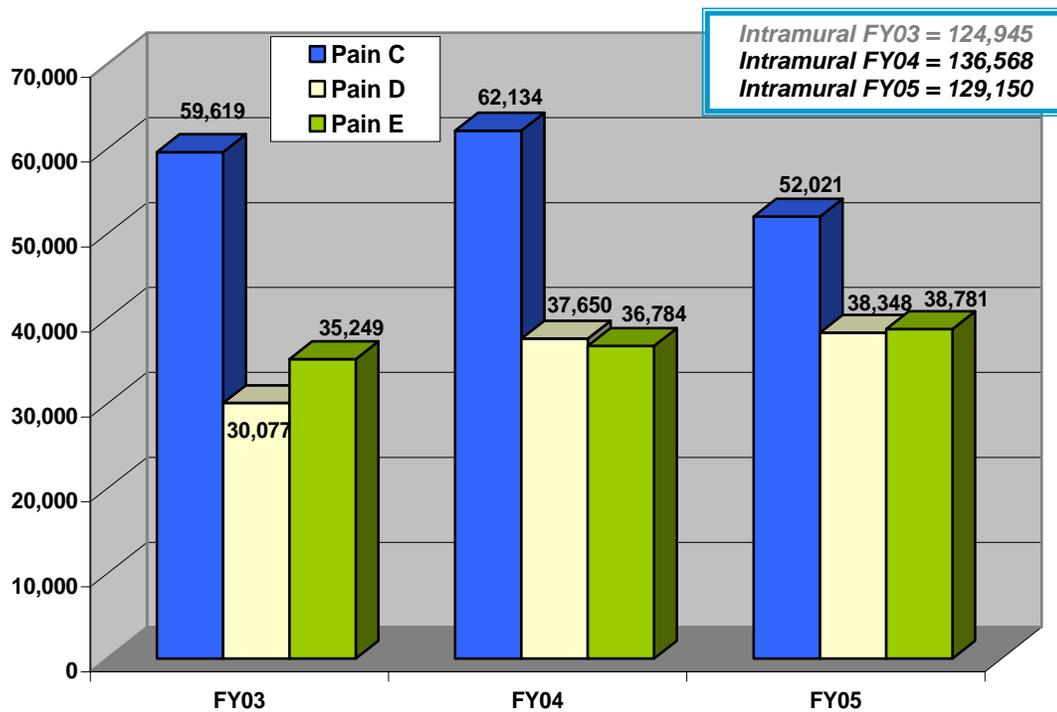


Figure 2-16 Intramural Animal Use by USDA Pain Category for FY04–FY05 (FY03 for comparison)

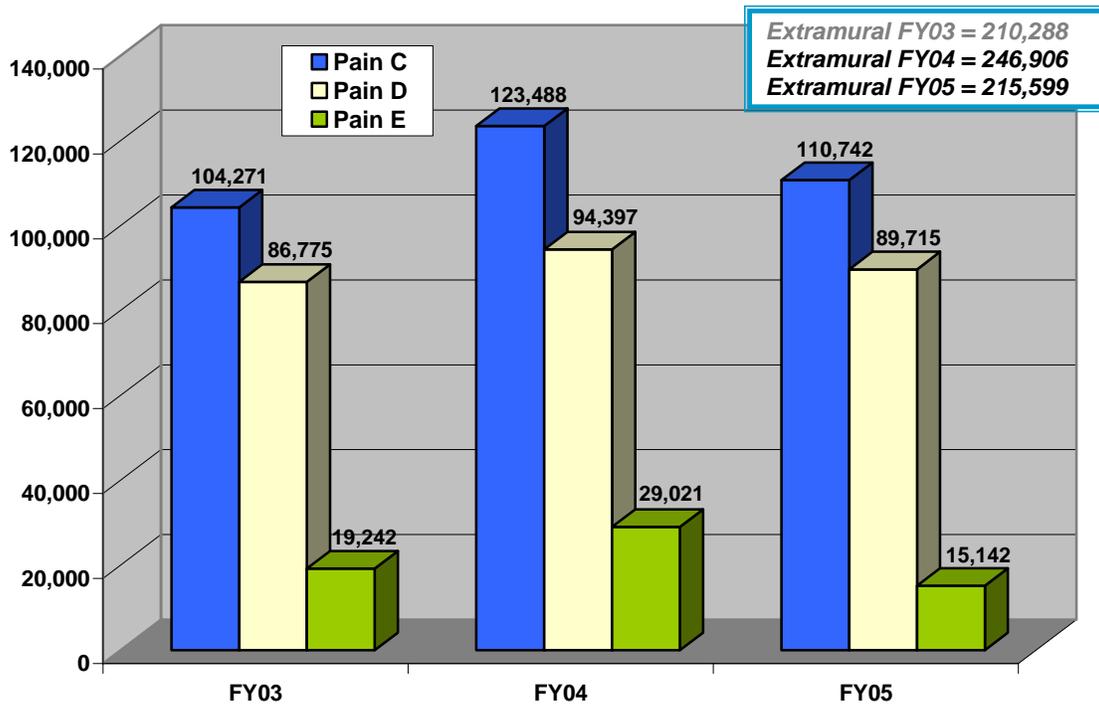


Figure 2-17 Extramural Animal Use by USDA Pain Category for FY04–FY05 (FY03 for comparison)

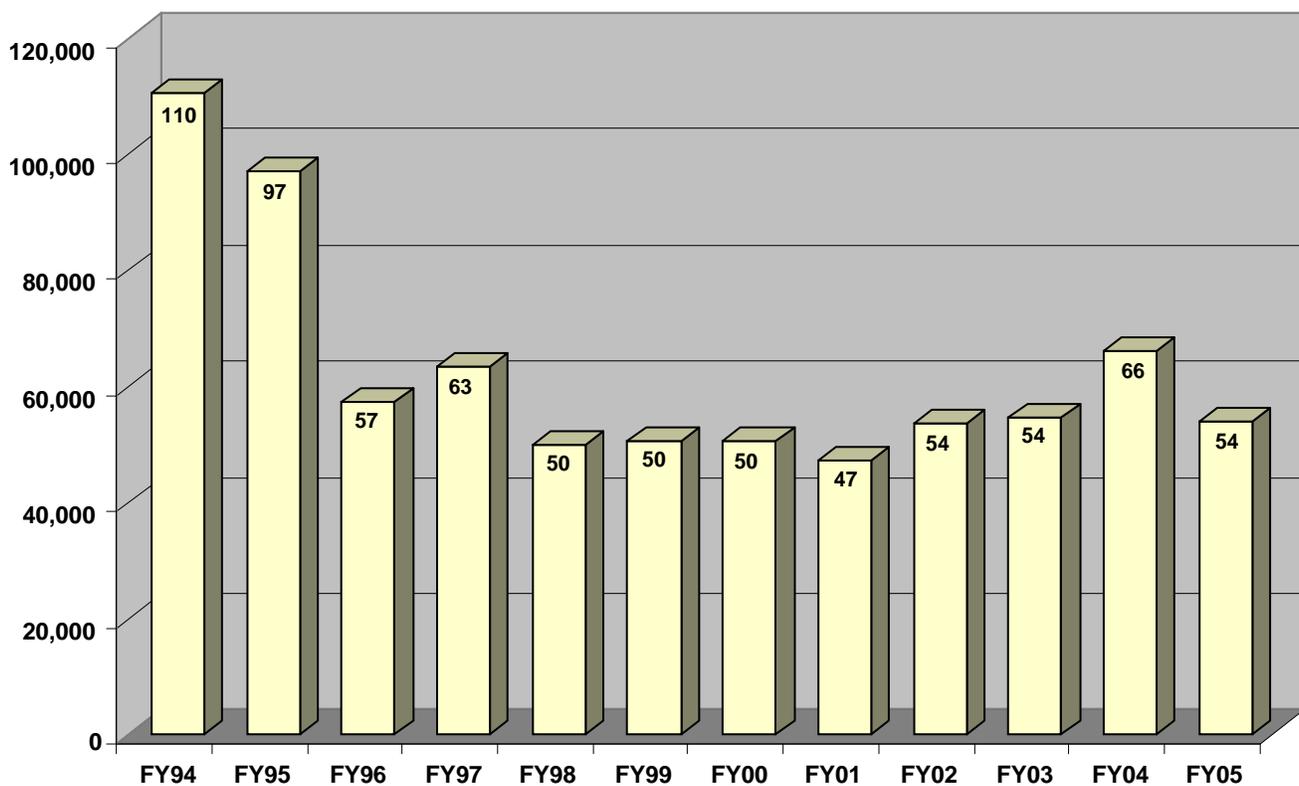


Figure 2-18 USDA Pain Category E by Fiscal Year

The majority (83%–84%) of FY04–FY05 DoD-supported research employing any species of animal was considered not painful to the animals involved. Around 34%–38% of all animals were not exposed to or involved in any potentially painful procedures (USDA Pain Category C). Between 34%–37% of all animals were given anesthesia or pain-relieving drugs to prevent pain or distress (USDA Pain Category D). In 16%–17% 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 except for a low spike in FY04 (Figure 2-18).

The words “potentially painful” reflect the fact that a Pain Category E classification is applied to any protocol wherein pain may be realized or cannot otherwise be fully assessed. For example, per USDA policy, a Pain Category E designation was used for more than 3,000 fish subjected to low-level toxicity studies. 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. Typically, a majority (60% in FY05) of animals used in potentially painful experiments were rodents. Other mammals accounted for 0.5% of animals in this pain category in FY05.

Figure 2-19 shows the numbers of animals used in Pain Category E by the animal use category. In FY05, 94% of the animals reported in USDA Pain Category E were used in medical studies (Category M). Of these, 65% 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 20% of all FY05 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, N, and T.

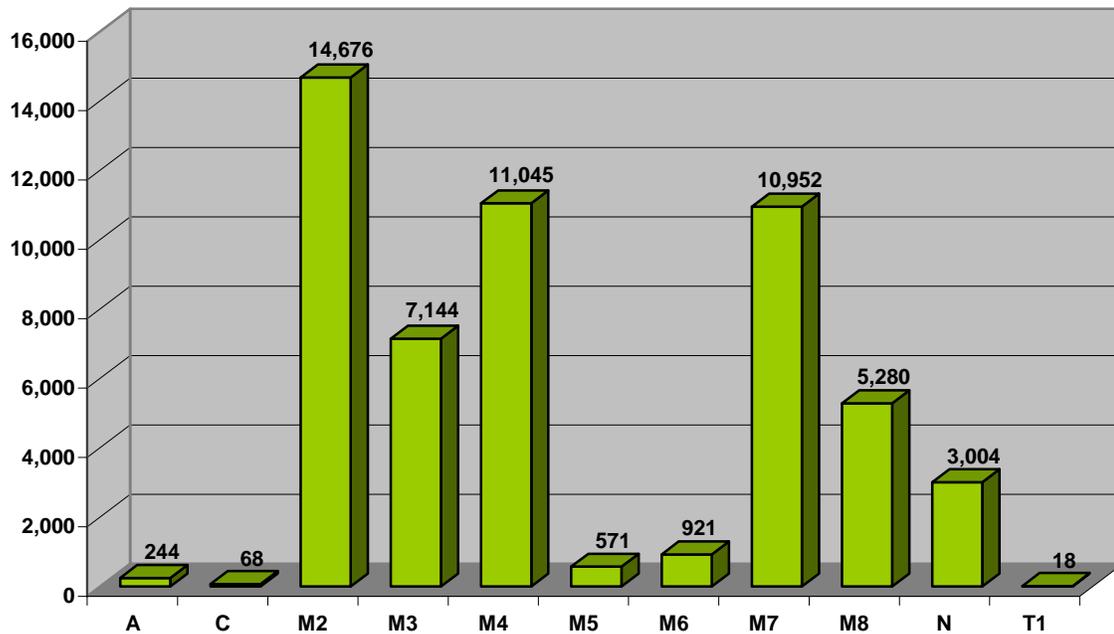


Figure 2-19 Number of USDA Pain Category E Animals by Animal Use Category (FY05)

A–Adjuncts/Alternatives to Animal Studies, C–Clinical Investigations, 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, N–Nonmedical RDT&E, T1–Training/Instructional

The DoD clearly has diverse, unique, and demanding RDT&E and training missions that provide 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 atmospheric pressure, and gravitational forces are threats to service members. 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 members from the threats encountered over the course of performing their missions.

SECTION 3

DoD INITIATIVES TO PROMOTE ALTERNATIVES TO USING 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 end points; 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 consideration and selection of alternatives to animals.

The DoD has established a variety of initiatives and targeted programs that are currently in place to responsibly promote alternative methods to replace, reduce, and refine the use of animals not only within but also outside of the department. 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.

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 in this section. 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-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 FY04–FY05, chemical and

biological defense projects totaling more than \$1.3 million 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. DoD organizations such as the U.S. Army Soldier and Biological/Chemical Command, 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, and the U.S. Air Force have sponsored or cosponsored conferences with organizations such as the U.S. Department of Health and Human Services, National Institute of Environmental Health Sciences (NIEHS); the Interagency Committee on Neurotoxicology; the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM); Xenogen Corporation; the Gillette Company; the Humane Society of the United States; DermTech International; the National Capital Area Chapter of the Society of Toxicology; and the Association of Government Toxicologists. These national and international meetings serve as scientific forums for the exchange of 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 more than 125 scientists from industry, government, and academia, *Alternative Toxicological Methods for the New Millennium*, 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.

3.2 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.

3.3 DOD PARTICIPATION IN FEDERAL ANIMAL ALTERNATIVE PROGRAMS

ICCVAM was established in 1997 by NIEHS in response to statutory mandates for NIEHS to recommend a process by which scientifically validated alternative methods could be accepted for regulatory use. Congress formalized ICCVAM in the ICCVAM Authorization Act of 2000 (42 USC 3851-3).

ICCVAM is a federal committee with representatives from 15 health research and regulatory agencies, including the DoD. ICCVAM's focus is to promote the development, validation, regulatory acceptance, and harmonization of new and revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness. ICCVAM gets support for test method evaluations, workshops, and peer reviews from the National Toxicology Program Interagency Center for the Validation of Alternative Methods. ICCVAM does not "accept" test methods but rather develops recommendations about the usefulness and limitations of the methods. Each agency then evaluates and makes acceptance decisions on the test method recommendations according to its own statutory requirements.

ICCVAM has had a number of successes. To date, ICCVAM has successfully reviewed more than 185 test methods (<http://iccvam.niehs.nih.gov/home.htm>) during its first 10 years of existence. ICCVAM recommendations have resulted in national and international adoption or endorsement of 10 new alternative tests for the 4 most commonly performed product safety tests used to determine if products used in the home and workplace can cause acute oral toxicity (poisoning), skin damage (irritation and chemical burns), allergic skin

reactions, and eye damage (irritation and blinding). These alternative test methods have significantly reduced the number of animals required for safety assessments and provided for improved welfare of animals used in safety evaluations.

ICCVAM and the DoD are committed to making good science-based decisions. ICCVAM follows a formal test method evaluation process that is transparent, scientifically rigorous, open to public inspection, and with all materials made publicly accessible. ICCVAM solicits nominations from the public and stakeholder organizations for scientists with a range of relevant expertise needed to conduct a thorough review of the scientific validity of a proposed test method. The ICCVAM evaluation process is very rigorous. ICCVAM is actively collaborating with our European and international counterparts.

ICCVAM has established a plan for progress. ICCVAM has launched a 5-year plan to further reduce, refine, and replace the use of animals in research and regulatory testing. A cornerstone of this plan is the formation of partnerships with industry and other national and international stakeholders to achieve measurable progress. ICCVAM also is emphasizing the use of new technologies to develop predictive systems that would be less reliant or not at all reliant on animals. To maximize the efficiency of this process, ICCVAM is working with federal agencies and other stakeholders to link research and development activities to the standardization and validation of alternative test methods that may be used in regulatory testing.

3.4 DOD EXPERTISE AND TRAINING PROGRAMS THAT PROMOTE ANIMAL ALTERNATIVES

In FY04–FY05, the DoD’s veterinary training programs yielded seven residency graduates and six 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, 3-year program culminating in specialty board eligibility for certification by the ACLAM. In August 1995, the DoD began a formal postgraduate master’s of public health program 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 development programs.

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

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 technician’s course and WRAIR workshop curriculum include formal training and information on animal use alternatives.

3.5 DOD IMPLEMENTATION OF THE 3RS

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.

The following examples of the 3Rs represent general alternative methods used by DoD facilities during FY04–FY05.

Replacement

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

Reduction

- Animal use protocols were subject to review by a biostatistician who addressed the animals used, study design, and statistical evaluation packages, and ensured that the minimum number of animals would be used to meet the specific scientific objectives.
- Pilot studies were used to refine techniques and define the animal model so that animal use could be kept to the minimum required for statistical significance.
- Sharing of animal tissues among investigators reduced animal use.
- Iterations of the experiments were combined when possible to reduce the number of control animals used.
- Collaboration between DoD investigators or instructors allowed 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 were collected simultaneously.
- Training sessions were designed to use the highest practical student-to-animal ratio.
- When possible, animals served as their own controls.
- Studies were deliberately phased so they continued only if warranted.

Refinement

- Parameters developed for early or alternative end points were used as experimental end points when possible.
- Animals were anesthetized before euthanasia to decrease stress.
- Moribund animals were humanely euthanized to prevent unnecessary pain or distress.
- Animals were 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 were taught to animal technicians, investigators, and research assistants to increase or ensure that a proper skill level was attained before starting a protocol.
- All advanced trauma life support training laboratory procedures were performed with animals under general anesthesia, and they were euthanized without regaining consciousness.

3.5.1 Research Protocol and IACUC Emphasis on Alternatives

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

using animals in research, education, testing, or training include all of the information contained in the DoD Standard Protocol Format, thereby requiring the alternatives information.

3.5.2 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. WRAIR was one of the first institutions to establish a policy that mandates environmental enrichment for research animals, a practice in widespread use at animal facilities today.

3.5.3 Specific Alternatives Employed During FY04–FY05

FY04–FY05 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, reliable, and validated procedures that can be performed without the use of animals. In addition, in cases where animal models cannot be completely replaced, investigators and veterinary staff 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.

The following list provides brief examples that are representative of the specific alternative methods used by DoD facilities during FY04–FY05.

Replacement

Replacement Using In Vitro Cell Cultures:

- In vivo models of schistosome growth and development were characterized toward defining and incorporating essential model requirements with the goal of developing an in vitro model for studying schistosome growth and development and reducing or eliminating animal use.
- In vitro cell cultures were used to test the feasibility of inhibiting RNA to block scar formation.
- Instead of measuring lethal toxin activity in mice, researchers developed a cell culture assay to determine the activity of the toxin on macrophages.
- The use of in vitro studies to measure biomarkers considerably reduced the use of mice.
- Researchers screened a novel formulation of a drug protecting against radiation exposure using in vitro tissue culture, searching for cytotoxic effects first, before administering it in vivo.
- In vitro cell cultures were used instead of mice for biochemical and microscopy studies of T cell activation.

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

- Instead of using rats, fish were used for the isolation of stem cells from the brain.
- Frogs replaced mammals in the study of toxicant effects on the development of reproductive and thyroid systems.
- Mice were used in research instead of the original species of monkey.
- The fathead minnow larva is the lowest vertebrate available for use in a study. Its larval nervous system is not fully developed at the time of testing, reducing pain processing.
- Fish are routinely used in toxicity studies.

Biochemical/Physical Methods and Other Technologies:

- Ophthalmology residents learned several techniques for the treatment of eyes using bovine eyes collected and purchased from a slaughterhouse.
- Nonanimal models were implemented in training protocols to minimize animal use to “hands-on” aspects of procedural training.

- Prior to live animal use, training participants received detailed instruction on procedures and techniques through didactic lectures, videotapes, and experience with pelvic trainer models including the use of plastic or foam tissues and/or organ models.
- Nonphysician health care providers (i.e., physician assistants, nurses, and medical technicians) were trained on various wound care and suturing techniques using pigs' feet, and the administration of intraosseous fluids was simulated by using chicken legs. Both were purchased from a local grocery store.
- Pigs' feet and oranges purchased from a local grocery store were used to teach skin biopsy, suturing techniques, and intraosseous fluid administration, replacing live animal use.
- Computer simulations such as the Simulab TraumaMan[®] manikin augmented live animal use in advanced trauma life support training programs.

Reduction

Substitution of Computer Simulation, Models, or Other Technologies:

- Manikins were used initially to teach intubation techniques.
- All samples of lipopolysaccharide used in a study were initially screened in a cell culture system for biological activity before being used in animals. This eliminated those test samples not capable of inducing TNF-alpha release in vertebrate cells.
- Cell cultures eligible for in vivo tumorigenic assay validation were prescreened in vitro for evidence of response to growth inhibition by potential therapeutic agents (MTT assay) and by soft agar clonogenic assay as surrogate markers for malignant potential.
- Cadavers were used to the maximum extent possible. In addition, inanimate training aids and models were used including the Koken[®] rat, suture boards, and stuffed infant socks.
- Classroom instruction is supported with illustrations, PowerPoint presentations, and videos.
- Endotracheal intubation, blood sample withdrawal, and intravenous and urinary catheter placement were practiced using simulation manikins prior to performing in vivo procedures. These additional training tools increased student skills, confidence, and accuracy when performing tasks, thereby reducing distress and discomfort to animal subjects.
- Human manikins were initially used to teach intubation, thereby reducing potential trauma to ferrets in the laboratory.
- Manikins were used to give students some experience in establishing airways, and "tie boards" were used to practice surgical knot tying to reduce the number of animals needed.
- Training protocols incorporated a mock circulatory system and used postmortem specimens. Also, archived records of procedures, hemodynamics, and angiographic and ultrasound/Doppler data were edited and made available as added training material.
- Simulation manikins were used to teach canine cardiopulmonary cerebral resuscitation, eliminating the use of live animals to teach this skill.
- Koken and PVC rat models with artificial blood vessels and nerves were purchased for the residents in training to practice on prior to using live rats, greatly reducing the number of rats needed for training.
- 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.

Experimental and Training Strategies:

- By using weight-bearing and non-weight-bearing surfaces in the same joint, the total number of goats needed for the study of implants was halved in studies of materials to heal cartilage in joints.
- Following veterinary training procedures, animals were euthanized before airway intubation training, and the heads were harvested and preserved for later use in providing Animal Care Specialist students realistic training in dental care and cleaning.
- If appropriate, after an experiment, animals may be given to another terminal study while maintained under strict anesthesia throughout.
- In medic training, four students were assigned to each goat, reducing the number of goats needed. After the laboratory procedure, limbs were harvested from the carcasses for use in teaching wound management to Animal Care Specialists.

- Historical controls were used, reducing the number of animals required.
- Mice, instead of ferrets, were initially used for training, reducing the number of ferrets required.
- Monkeys that could no longer be used for biomedical purposes were retired to the regional primate center as breeders instead of being euthanized.
- Multiple measurements were recorded from each animal to maximize the amount of data being acquired from each and reduce total animal numbers. Baseline data from a previous study were used for several comparisons to be made, eliminating the need for duplication.
- Control group data were generated once and subsequently used in multiple experiments.
- Data from previous experiments were used to adjust the time of antibody administration, dose of antibody administered, and optimal time for sample collection. This reduced the number of animals needed to obtain meaningful results.
- Animal use was reduced by using primary cell cultures stimulated by growth factors.
- To reduce the numbers of animals used in testing vaccine constructs, plasmids were first tested in vitro for expression of the protein product.
- An increased number of mouse tissue samples per experiment was taken to cut down on the number of animals required. For example, instead of analyzing just liver and spleen to measure bacterial loads, mesenteric lymph nodes, fecal pellets, caecum, and ileum also were examined.
- Rat cadavers from rodent handling classes were used to practice probe placement before surgery was performed on live animals.
- Dogs used in the training of canine anesthesia also were used in the instruction of care, handling, and management classes.

Refinement

Reducing Pain and Distress:

- Indwelling jugular catheters were used to collect serial blood samples, minimizing distress, potential infection, and injury from repeated needle sticks.
- Animals were sedated during hearing testing in the laboratory before and after exposure to sound.
- Central intravenous access, rather than a surgical procedure, was used with prolonged anesthesia to minimize the invasiveness of the procedure and reduce pain and discomfort to the animals.
- Efforts were made to minimize pain and suffering by using smaller needles for intravenous injections.
- Doses of anesthesia were both increased and enhanced with barbiturates for maximizing analgesia.
- Surgical procedures were developed to decrease stress and discomfort to animals. Refined and less invasive techniques were used to make the implantation of cranial electroencephalogram leads less invasive. The animals were under anesthesia for less time, recovery was shorter, and there were reduced signs of postoperative pain.
- General anesthesia was used to reduce any stress during manual restraint procedures.
- Real-time telemetry was used to monitor core body temperature, intrathoracic pulmonary pressure, and electrocardiogram (ECG) in study animals, reducing the need to remove the animals from their enclosures, and thereby lowering handling stress and injury to handlers or animals. Doppler microwave activity monitoring receivers were further used to measure animal physical activity within the enclosures.
- Surface and subdermal electrodes were used for long-term electrophysiological follow-up after nontoxic exposure to a chemical agent.
- In infectivity studies, animals were euthanized at the first signs of infection, implementing early humane study end points.
- Use of the modified Buehler method eliminated adjuvant injections, allowing the animals to roam freely in their cages during the test period instead of being restrained.
- Rodents are handled daily to acclimate them to handling and reduce stress.
- Colony animals received annual physical exams, routine dental cleanings and radiographs, and occasional emergency care to optimize health and well-being.
- The use of infrared imaging to assess blepharospasm eliminated the need for more invasive procedures.
- Nylabones[®] were provided for environmental enrichment when rats were individually housed.

- All animals were provided with moistened food and water immediately after surgery until they were ambulatory enough to attain food and water from cage-top dispensers.

Increased Training for Research Personnel to Improve Skills:

- Training programs were developed to teach research personnel the technical skills necessary to properly manage and humanely handle animals during research procedures.
- Technique-oriented videotapes and nonanimal models were used to improve confidence and proficiency before training with live animals.
- The Koken rat model was used to practice handling, restraint, and gastric lavage prior to practicing on live animals, thus reducing the number of attempts necessary for students to learn specific techniques.

3.5.4 Alternatives Undergoing Development During FY04–FY05

As an ongoing process, the DoD is continuously developing alternatives. The following are examples of alternatives that were reported as in development by the DoD during FY04–FY05. These are only a sample of the alternatives being developed.

Replacement

Replacement Using Models or In Vitro Cell Cultures:

- Researchers were establishing an in vitro thermal pretreatment and injury challenge model to allow for the rapid evaluation of pharmacologic agents against thermal injury. This model will generate 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 *Escherichia coli* was urgently needed to design and test therapies. To address this need, researchers began developing a three-dimensional human intestinal organoid system that could be used in certain experiments as a mouse model alternative.
- A three-dimensional human bladder organoid system was under development that could be used in certain experiments as an alternative to a currently used mouse model.

Nonmammalian Species or Species Lower on the Phylogenetic Scale:

- Pharmacological studies employing visually stimulating testing and cognitively challenging behavioral tests were being adapted for implementation in mice and rats instead of NHPs.
- A pilot study was conducted to discover and clone genes that could serve as toxicity markers using the Japanese medaka fish as an animal model.

Reduction

Substitution of Computer Simulation, Models, or Other Technologies:

- In vaccine development, the identification of immunological target epitopes will be conducted using computer searching. This will eliminate trial and error screening employing live animals.
- The use of membrane blood feeding was being developed further as an alternative to using mice to maintain mosquito and mite colonies used to conduct studies on the transmission of malaria, dengue, and scrub typhus.
- Investigation of gene expression profiles in cell cultures exposed to toxic chemicals was 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 ultimately will reduce the number of animals necessary for current and future studies while increasing the amount and quality of information obtained per animal.

Utilization of Alternative Biological Testing Methods:

- Preliminary studies using research-grade plasmids reduced the number of animals required to test vaccine lots.
- Boosting the immune system of mice with stimulants was 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 was being developed for NHPs by engaging them in behavioral interaction emulating the essential features of natural foraging. The results will be used to further refine the environmental conditions of captive NHPs and ensure their psychological well-being.
- Novel strategies and methods for improved environmental enrichment were being evaluated for many different animals.
- Researchers were testing and validating an alternative, rapid method for taking intraocular pressure measurements that requires no manual animal restraint, use of anesthetics, or sedation.

Reduce Pain and Distress:

- Studies were conducted to develop methods to collect free-catch urine samples and avoid the invasive procedure of cystocentesis for urine collection.
- Efforts were under way to identify alternate markers for the successful development of an immune response.

3.6 SUMMARY OF INITIATIVES TO USE ALTERNATIVES TO ANIMALS

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 FY04–FY05, there was significant evidence of the DoD’s aggressive pursuit to develop alternatives to replace, reduce, and refine the use of animals. In addition to these developmental efforts, animal use data for FY04–FY05 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 FY05 showed an increase in NHP use, the use of dogs and cats has decreased by 97% and 73%, respectively, relative to FY94. Together, these 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, the 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.

SECTION 4

CONCLUSION

It remains essential to use animals in DoD RDT&E and training programs to protect the health and lives of military personnel. Alternatives to animal use will continue to be vigorously sought and applied. Until validated alternatives exist that simulate the complex interactions of organ, tissue, cell, disease agents or processes, and environment, the continued judicious use of animals in DoD programs is 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.

APPENDIX – 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
APHIS	Animal and Plant Health Inspection Service
AWA	Animal Welfare Act
CDMRP	Congressionally Directed Medical Research Programs
DDR&E	Director, Defense Research and Engineering
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
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