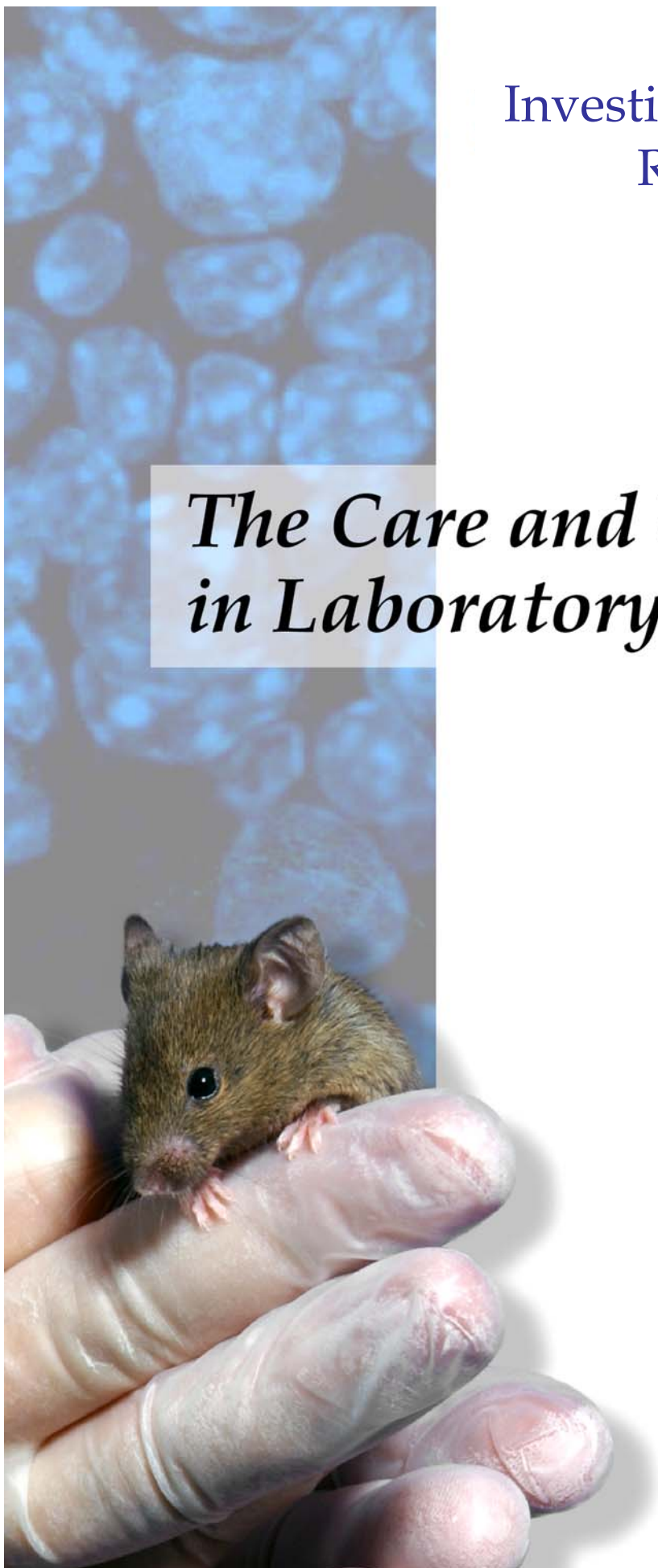


Investigator and Animal User  
Reference Manual

*The Care and Use of Animals  
in Laboratory Research*

2007 Revision

 **NCI** Frederick



# NCI-Frederick Investigator and Animal User Reference Manual

## *Care and Use of Animals in Laboratory Research*

### TABLE OF CONTENTS

<b>Introduction</b> .....	4
<b>Contact Information</b> .....	5
<b>Regulations and Guidelines</b>	
<i>Animal Welfare Act</i> .....	7
<i>PHS Policy on the Humane Care and Use of Laboratory Animals</i> .....	7
<i>U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training</i> .....	8
<i>Guide for the Care and Use of Laboratory Animals</i> .....	9
<i>Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research</i> .....	9
<i>AVMA Guidelines on Euthanasia</i> .....	9
<i>Endangered Species Act</i> .....	9
<b>Professional Organizations Involved in Biomedical Research</b> .....	10
<b>NCI-Frederick Animal Care and Use Committee</b> .....	14
<b>Painful Procedures and Euthanasia</b>	
<i>Guidelines for Pain/Distress Classification</i> .....	16
<i>Alternatives to Animal Models</i> .....	18
<i>Surgery</i> .....	20
<i>Anesthesia and Analgesia</i> .....	23
<i>Euthanasia</i> .....	29
<i>DEA and Controlled Substances</i> .....	36
<b>Animal Health and Disease</b>	
<i>Laboratory Animal Medicine Program</i> .....	37
<i>Zoonoses</i> .....	37
<i>Signs of Animal Disease</i> .....	38
<i>Animal Procurement</i> .....	38
<i>Quarantine, Stabilization, and Isolation</i> .....	39
<i>Rederivation</i> .....	40
<i>Separation of Species, Source, and Health Status</i> .....	40
<i>Surveillance, Diagnosis, Treatment, and Control</i> .....	40
<i>Animal Health Programs</i> .....	41
<i>Diagnostic Resources</i> .....	42

## TABLE OF CONTENTS - CONTINUED

### **Animal Holding and Technical Support Program**

<i>General Information</i> .....	43
<i>Security</i> .....	43
<i>Overview of Laws and Regulations</i> .....	44
<i>Space Recommendations for Laboratory Animals</i> .....	44
<i>Minimum Space Requirements for Laboratory Animals</i> .....	45

### **Safety and Environmental Compliance**

<i>Use of Hazardous Agents at the NCI-Frederick</i> .....	46
<i>Biohazards</i> .....	46
<i>Chemical Hazards</i> .....	46
<i>Fire, Life Safety, and Physical Hazards</i> .....	46
<i>Radiological Hazards</i> .....	46
<i>Recombinant DNA</i> .....	47
<i>Safeguards</i> .....	47
<i>Facility/Engineering Controls</i> .....	48
<i>Safety Inspections</i> .....	48
<i>Exposure Monitoring for Employees Using Hazardous Agents</i> .....	49

<b>Occupational Health Program</b> .....	49
--	----

<b>References</b> .....	50
-------------------------	----

<b>ACUC Policies and Guidelines</b>	<a href="http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/main.asp">http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/main.asp</a>
-------------------------------------	---

## *Introduction*

These materials were prepared to supplement the online NCI-Frederick Animal Care and Use training courses. Any individuals working with live animals should carefully read these materials and retain them for future reference.

Public Health Service Policy on Humane Care and Use of Laboratory Animals, mandated by the Health Extension Act of 1985, and the Animal Welfare Act require training or instruction to scientists, animal technicians, and other personnel involved in animal care, treatment, or use. The training or instruction must include information on: a) the humane practice of animal care and use; b) the concept, availability, and use of research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress [as well as the services available to facilitate pertinent literature searches]; (c) proper use of anesthetics, analgesics, and tranquilizers; (d) mechanisms for reporting animal care and use concerns; and (e) occupational health and safety.

The NCI-Frederick Animal Care and Use Committee utilizes the online NCI-Frederick Animal Care and Use introductory training course, the supplemental online refresher training course, as well as web resources to fulfill these regulatory requirements. All individuals working with animals have a responsibility to the institution and the general public to conduct humane and ethical research that balances the risks, burdens, and benefits to include sound research design that provides a benefit to the health or welfare of humans or other animals. The NCI-Frederick strongly encourages all investigators to consider alternatives prior to initiating an animal research project. If alternatives are not feasible for your research study, you are required to ensure humane care, alleviate pain and distress, and to implement humane endpoints.

All individuals working with animals are responsible for the well-being of the animals under their care and required to promptly report concerns or neglect to the NCI-Frederick Animal Care and Use Committee [or anonymously to any individuals listed at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/contact.asp>]. Please refer to the notice for Reporting Animal Care and Use Concerns disseminated by the NCI-Frederick Associate Director [<http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/ACUCConcerns.pdf>] to ensure our commitment to humane care and use.

Additional information on the proper care, handling, and use of laboratory animals can be obtained by contacting the Chair or Coordinator of the NCI-Frederick Animal Care and Use Committee, the Director of the Laboratory Animal Sciences Program, the Deputy Director of the Laboratory Animal Sciences Program, the Head of Laboratory Animal Medicine, or the LAM veterinary staff. The NCI-Frederick Animal Care and Use Committee website can be found at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/main.asp>

*NCI-Frederick LASP and ACUC Contact Information*

Name	Title	Phone/Email
Dr. Craig Reynolds	Associate Director, NCI [Institutional Official]	301-846-1302 reynoldsc@mail.ncifcrf.gov
Dr. Melinda Hollingshead	Chair, NCI-Frederick ACUC	301-846-5608 hollingm@mail.nih.gov
Dr. Hendrick Bedigian	Director, Laboratory Animal Sciences Program	301-846-1542 bedigiah@mail.nih.gov
Dr. Bishop Curry	Deputy Director, Laboratory Animal Sciences Program	301-846-1542 curryb@mail.nih.gov
Dr. Lionel Feigenbaum	Director, Scientific Support, Laboratory Animal Sciences Program Head, Transgenic Mouse Model and Knock-Out Mouse Services	301-846-1696 feigenbaum@mail.ncifcrf.gov
Dr. Jeanne Herring	Head, Laboratory Animal Medicine Attending Veterinarian Manager, Receiving and Quarantine	301-846-5195 herringj@ncifcrf.gov
Ms. Michelle Gottholm Ahalt	NCI-Frederick ACUC Coordinator	301-846-7544 ahaltgottholmm@mail.nih.gov
Dr. Scott Keimig	Manager, Occupational Safety	301-846-1906 keimig@mail.ncifcrf.gov
Mr. Troy Cregger	Receiving and Quarantine	301-846-1133 tcregger@ncifcrf.gov
Mr. Pete Gorelick	Animal Health Diagnostic Laboratory	301-846-1134 gorelickp@mail.ncifcrf.gov
Dr. Lionel Feigenbaum	MMHCC Repository Speed Congenics	301-846-1696 feigenbaum@mail.ncifcrf.gov
Dr. Joseph Kalen	Director, Small Animal Imaging Program	301-846-5283 kalenj@mail.nih.gov
Mr. Eleazar Vega-Valle	Cell Culture & Tumorigenicity Laboratory	(301) 846-7233 evegavalle@ncifcrf.gov
Ms. Parirokh Awasthi	Cryopreservation and Assisted Reproduction Laboratory	301-846-1864 pawasthi@ncifcrf.gov
Dr. Miriam Anver	Veterinary Pathology	301-846-1281 manver@ncifcrf.gov
Dr. Lawrence Sternberg	Molecular Pathology Histotechnology	301-846-6881 lsternberg@ncifcrf.gov

*NCI-Frederick Facility Manager Contact Information*

Facility	Manager	Phone/Email
APA	Cliff Hubbard	301-846-1203 hubbardc@mail.ncifcrf.gov
429	Dr. Jeanne Herring Troy Cregger	301-846-5195 301-846-1133
539-1CB	Terri McLellan	301-846-5059 lmclellan@ncifcrf.gov
539-1CC	Mark Shrader	301-846-1270 mwshrader@mail.ncifcrf.gov
539-2	Mark Shrader	301-846-1270 mwshrader@mail.ncifcrf.gov
538	Mark Shrader	301-846-1270 mwshrader@mail.ncifcrf.gov
550	Gene Oliver	301-846-5577 eoliver@ncifcrf.gov
553 Small Animal Imaging Facility	Dr. Joseph Kalen	301-846-5283 kalenj@mail.nih.gov
567	Jim Stull	301-846-1680 jstull@ncifcrf.gov
571	JanNean Williams	301-846-1177 jwilliams@ncifcrf.gov
1022 and 1048	Gene Oliver	301-846-5577 eoliver@ncifcrf.gov
1023, 1036, 1037, and 1049	Mark Shrader	301-846-1270 mwshrader@mail.ncifcrf.gov

## *Regulations and Guidelines*

### **ANIMAL WELFARE ACT**

The Animal Welfare Act of 1966 (P.L. 89-544), as amended by the Animal Welfare Act of 1970 (P.L. 91-579) and the 1976 Amendments to the Animal Welfare Act (P.L. 94-279), contains provisions to prevent the sale or use of animals that have been stolen; prohibit animal fighting ventures; and insure that animals used in research, for exhibition, or as pets receive humane care and treatment. The law provides for regulating the transport, purchase, sale, housing, care, handling, and treatment of such animals. The Act does not cover rats and mice.

Regulatory authority under the Animal Welfare Act is vested in the Secretary of the U.S. Department of Agriculture (USDA) and implemented by USDA's Animal and Plant Health Inspection Service (APHIS). APHIS is responsible for regulating not only research facilities, but also animal dealers, exhibitors (including zoos, aquariums, circuses, etc.) and intermediate handlers of animals, including air and truck lines. Rules and regulations pertaining to implementation of the Act are published in the Code of Federal Regulations (CFR), Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1, 2, and 3.

The Improved Standards for Laboratory Animal Act, a part of the 1985 Farm Bill, was enacted in Public Law 99-198. The new law amends the Animal Welfare Act effective December 1986, and requires the Secretary of Agriculture to promulgate new standards for the care, treatment, and use of laboratory animals, and to establish an information service at the National Agricultural Library. It also stipulates that the U.S. Department of Agriculture (USDA) shall inspect research facilities at least once a year, and that each facility must provide reports that verify compliance, train personnel involved with animal care, and establish an institutional animal committee to conduct semi-annual reviews.

The Animal Welfare Act covers all research facilities in the U.S. that use laboratory animals in basic and biomedical research, education, and product safety testing. In contrast, Public Health Service Policy (see below) affects only those institutions receiving PHS funding.

Copies of this act can be found at <http://www.aphis.usda.gov/ac/awapdf.pdf>. Related guidance can be found in the USDA Animal Care Policy Manual at <http://www.aphis.usda.gov/ac/polmanpdf.html>.

### **PUBLIC HEALTH SERVICE REGULATIONS**

The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions was updated in 1985. In the policy statement, the PHS endorses the U.S. government "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Education" (reprinted below), which were developed by the Interagency Research Animal Committee. The PHS policy implements and supplements these principles.

The Health Research Extension Act of 1985, Public Law 99-158, revising and extending authorities of the National Institutes of Health (NIH) under the Public Health Service Act, requires the Director of NIH to establish guidelines for the care and use of laboratory animals and requires recipients of NIH funds to provide assurances of their compliance with these guidelines and to have institutional animal committees. In addition, the law provides that the Director of the NIH must, by October 1986, develop a plan for research and training in valid alternatives to animal models, in methods to reduce the number of animals used, and in methods to minimize any pain and distress animals may experience. Public Health Service regulations cover rats and mice.

The NIH Office for Laboratory Animal Welfare (OLAW) has published the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, revised as of September 1986, incorporating the changes in the Public Health Service Act mandated by the Health Research Extension Act of 1985, Public Law 99-158.

This policy states, "All applications and proposals for award, which are either submitted to the PHS on or after November 1, 1986, or being conducted on or after July 1, 1987, must meet the requirements of the PHS Policy as amended. Institutions which currently have an approved or provisionally acceptable Animal Welfare Assurance on

file with the OLAW must submit to OLAW by July 1, 1987, a document in the form of an appendix or amendment which states the changes that the institution has made to conform to the amended Public Health Service Policy."

As required by the new law, major revisions are:

- The Policy will now apply to research that the PHS conducts intramurally.
- The Institutional Animal Care and Use Committee (ACUC) will be appointed by the chief executive officer of the institution.
- The institution's assurance must include a synopsis of the training or instruction made available to scientists, animal technicians and other personnel involved in animal care, treatment or use.
- The ACUC now must inspect and prepare reports on all of the institution's animal facilities (including satellite facilities) at least twice, instead of once, each year. The reports must be maintained by the institution and made available to OLAW upon request. AAALAC accredited facilities (Category 1) now must comply with this requirement since the law makes no distinction for them.
- The ACUC, through the Institutional Official, must submit written annual reports to OLAW updating the institution's assurance. These reports now must include minority views filed by members of the committee.

Copies of this policy can be found at <http://grants.nih.gov/grants/olaw/references/phspol.htm>

## U.S. GOVERNMENT PRINCIPLES FOR THE UTILIZATION AND CARE OF VERTEBRATE ANIMALS USED IN TESTING, RESEARCH AND TRAINING

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

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



appropriate review group such as an institutional animal research committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

## **GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS**

The *Guide for the Care and Use of Laboratory Animals* was originally prepared by the Institute for Laboratory Animal Resources of the National Research Council for NIH in 1963. These guidelines, which provide information on the care and use of laboratory animals in research, are not legally binding regulations. However, a NIH grantee must make a commitment to follow these recommendations in order to be eligible for a NIH Grant/Contract. These recommendations cover physical construction of animal facilities, husbandry, veterinary care, sanitation, qualifications of personnel, and other aspects of animal care. The *Guide* has been revised several times since 1963, with the latest in 1996.

*Copies of this guide can be found at <http://www.nap.edu/readingroom/books/labrats/>*

## **GUIDE FOR THE CARE AND USE OF MAMMALS IN NEUROSCIENCE AND BEHAVIORAL RESEARCH**

Expanding on the *Guide for the Care and Use of Laboratory Animals*, this publication provides current best practices for animal care and use and discusses how applicable regulations and guidelines can be applied to neuroscience and behavioral research. It encourages the use of professional judgment and careful interpretation of regulations and guidelines to develop performance standards that ensure animal well-being and high-quality research.

*Copies of this guide can be found at <http://www.nap.edu/books/0309089034/html/>*

## **AVMA GUIDELINES ON EUTHANASIA [2007]**

This report is intended for use by members of the veterinary profession who carry out or oversee the euthanasia of animals. Although the report may be interpreted and understood by a broad segment of the general population, a veterinarian should be consulted in the application of these recommendations. The practice of veterinary medicine is complex and involves diverse animal species. Whenever possible, a veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little species-specific euthanasia research has been done. Although interpretation and use of this report cannot be limited, the panel's overriding commitment is to give veterinarians guidance in relieving pain and suffering of animals that are to be euthanized. The recommendations of this report are intended to serve as guidelines for veterinarians who must then use professional judgment in applying them to various settings where animals are to be euthanized.

*Copies of this guideline can be found at [http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf)*

## **ENDANGERED SPECIES ACT**

The Endangered Species Act of 1973 (P.L. 93-205; 87 Statute 884) became effective on December 28, 1973, supplanting the Endangered Species Conservation Act of 1969 (P.L. 91-135; 83 Statute 275). The new law seeks "to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species, and to take such steps as may be appropriate to achieve the purposes of the treaties and conservation of wild flora and fauna worldwide."

Regulatory authority under the Endangered Species Act is vested in the Secretary of the U.S. Department of the Interior (USDI) and implemented by USDA's Fish and Wildlife Service. Implementing rules and regulations are published in the CFR, Title 50 (Wildlife and Fisheries), Chapter 1 (U.S. Fish and Wildlife Service, Department of Interior), Subchapter B, Part 17 (Endangered and Threatened Wildlife and Plants).

## *Professional Organizations Involved in Biomedical Research*

### **AAALAC**

The American Association for Accreditation of Laboratory Animal Care – International (AAALAC) is a nonprofit corporation formed in 1965 by leading U.S. scientific and educational organizations to promote high quality animal care and use through a voluntary accreditation program. Any institution maintaining, using, importing, or breeding laboratory animals for scientific purposes is eligible to apply for AAALAC accreditation. The animal care facilities of applicant institutions are visited and thoroughly evaluated by experts in laboratory animal science, who submit a detailed report to the Council on Accreditation. The council reviews applications and site visit reports, using the guidelines in the *Guide for the Care and Use of Laboratory Animals*, to determine whether full accreditation should be granted. Accredited facilities are required to submit annual reports on the status of their animal facilities. Site revisits are conducted at intervals of 3 years or fewer. The Council on Accreditation reviews the annual and site revisit reports to determine whether full accreditation should be continued.

Fully accredited animal care facilities receive a certificate of accreditation and are included on a list of such facilities published in the association's Activities Report. Full accreditation by AAALAC is accepted by the National Institutes of Health as partial assurance that the animal facilities are in compliance with PHS policy.

The NCI-Frederick has been accredited by the AAALAC since 1975.

*Additional information on the AAALAC can be found at <http://www.aaalac.org/>*

### **AALAS**

The American Association for Laboratory Animal Science (AALAS) is an organization made up of individuals and institutions professionally concerned with the production, care, and use of laboratory animals. It provides a means for collection and exchange of information on all phases of animal care and management. The association meets annually and publishes *Laboratory Animal Science* (a bimonthly journal), *Contemporary Topics in Laboratory Animal Science* and other documents.

The AALAS Animal Technician Certification Board provides a means of developing uniform requirements for technician training by defining qualifications, preparing and approving examinations for training programs, and certifying successful candidates.

*Additional information on the AALAS can be found at <http://www.aalas.org/>*

### **ACLAM**

The American College of Laboratory Animal Medicine (ACLAM) is a specialty board recognized by the American Veterinary Medical Association (AVMA). It was founded in 1957 to encourage education, training, and research; to establish standards of training and experience for qualification; and to certify, by examination, qualified laboratory animal specialists as diplomates. To achieve these goals, the college seeks to interest veterinarians in furthering both training and qualifications in laboratory animal medicine.

The annual ACLAM Forum is a major continuing education meeting. ACLAM also meets and sponsors programs in conjunction with the annual meetings of AVMA and the American Association for Laboratory Animal Science. It emphasizes and sponsors continuing-education programs; cosponsors symposia; cosponsors about 30 autotutorial programs on use, husbandry, and diseases of animals commonly used in research; and has produced 14 volumes on laboratory subjects, such as *The Laboratory Rat* and *The Mouse in Biomedical Research*.

*Additional information on the ACLAM can be found at <http://www.aclam.org/>*

## **ARENA**

The Applied Research Ethics National Association (ARENA) is a national membership organization for professionals concerned with issues relating to the protection of human subjects, the humane care and treatment of animals, scientific misconduct, ethical decision-making in healthcare, and other ethical issues pertaining to biomedical and behavioral research. ARENA's mission is the promotion of networking among its members, the development of educational activities, the resolution and/or amelioration of mutual problems, and the professional advancement of its members in order to enhance the ethical conduct of research and medicine. Members of ARENA include IRB and ACUC administrators and members, government representatives, researchers, veterinarians, hospital administrators, patient advocacy and animal welfare representatives, clinical and legal professionals, and others interested in bioethics.

*Additional information on the ARENA can be found at <http://www.primr.org/arena.html>*

## **ASLAP**

The American Society of Laboratory Animal Practitioners (ASLAP), founded in 1966, is open to any graduate of a veterinary college accredited or recognized by the American Veterinary Medical Association (AVMA) or Canadian Veterinary Medical Association (CVMA) who is engaged in laboratory animal practice and maintains membership in the AVMA, CVMA, or any other national veterinary medical association recognized by the AVMA. Its purpose is to disseminate ideas, experiences, and knowledge among veterinarians engaged in laboratory animal practice through education, training, and research at both pre- and postdoctoral levels. Two educational meetings are held annually in conjunction with the annual meetings of the AVMA and AALAS.

*Additional information on the ASLAP can be found at <http://www.aslap.org/>*

## **AVMA**

The American Veterinary Medical Association (AVMA) is the major national organization of veterinarians. Its objective is to advance the science and art of veterinary medicine, including its relationship to public health and agriculture. The AVMA is the recognized accrediting agency for schools and colleges of veterinary medicine. It sponsors specialization in veterinary medicine through the formal recognition of specialty certifying organizations, including the American College of Laboratory Animal Medicine. The AVMA Committee on Animal Technician Activities and Training accredits 2-year programs in animal technology at institutions of higher education throughout the United States. A list of accredited programs and a summary of individual state laws and regulations relative to veterinarians and animal technicians is available from the AVMA.

*Additional information on the AVMA can be found at <http://www.avma.org/>*

## **AWIC**

The Animal Welfare Information Center (AWIC) provides information for improved animal care and use in research, teaching, and testing.

*Additional information on the AWIC can be found at <http://www.nal.usda.gov/awic/index.html>*

## **CAAT**

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) has worked with scientists since 1981 to find new methods to replace the use of laboratory animals in experiments, reduce the number of animals tested, and refine necessary tests to eliminate pain and distress.

*Additional information on the CAAT can be found at <http://caat.jhsph.edu/>*

## **FBR**

The Foundation for Biomedical Research (FBR) was established in 1981 to take positive action to preserve the freedom of the scientific community to conduct biomedical research. FBR, a nonprofit educational organization provides the media and the public with accurate information about humane and responsible animal research.

Over the last three years, the Foundation has articulated the necessity for animal research in many forums and through a variety of media vehicles. More important, it has become the foremost resource in the nation for information on this critical subject, and has established a formal opposition to animal rights activists who formerly went unchallenged.

The Newsletter of the Foundation is published several times annually and provides information on FBR activities and educational materials, federal and state legislation, biomedical advances resulting from animal experimentation, and animal rights/welfare activities.

*Additional information on the FBR can be found at <http://www.fbresearch.org/>*

## **ICLAS**

The International Council for Laboratory Animal Science (ICLAS) is a non-governmental organization for international cooperation in laboratory animal science. It promotes international collaborative activities, aids development of internationally acceptable standards for care and use of laboratory animals, collects and disseminates information about laboratory animals, sponsors scholarships for education and training, and fosters programs to aid laboratory animal science in developing nations. The ICLAS Bulletin is issued every Spring and Autumn.

*Additional information on the ICLAS can be found at <http://www.iclas.org/>*

## **ILAR**

The Institute of Laboratory Animal Resources (ILAR) was founded in 1952 under the auspices of the National Research Council. A component of the Commission on Life Sciences, ILAR serves as a coordinating agency and a national and international resource for compiling and disseminating information on laboratory animals, promoting education, planning and conducting conferences and symposia, surveying existing and required facilities and resources, upgrading laboratory animal resources, and promoting high-quality, humane care of laboratory animals in the United States.

*Additional information on the ILAR can be found at <http://dels.nas.edu/ilar/>*

## **NABR**

The National Association for Biomedical Research (NABR), a nonprofit organization, was established in 1979 and consolidated in 1985 with the National Society for Medical Research. NABR membership includes more than 300 institutions including universities, medical and veterinary schools, teaching hospitals, voluntary health agencies, academic and professional societies, as well as pharmaceutical, laboratory animal breeders, and other research-intensive companies.

NABR supports the responsible use and humane care and treatment of laboratory animals in research, education and product safety testing; minimizing the number of animals used for research and testing; minimizing pain or

distress animals may experience; and the development and employment of alternatives to the use of live animals wherever feasible.

The Association recognizes that it is not possible, now or in the foreseeable future, to completely replace the use of animals and that the study of whole, living organisms is an indispensable element of biomedical research and testing that benefits all animals.

*Additional information on the NABR can be found at <http://www.nabr.org/>*

## **OLAW**

The Office of Laboratory Animal Welfare (OLAW) develops and monitors, as well as exercises compliance oversight relative to PHS Policy on Humane Care and Use of Laboratory Animals involved in research conducted or supported by any component of the Public Health Service. OLAW coordinates appropriate PHS regulations, policies, and procedures both within PHS and in coordination with other Departments and Agencies in the Federal Government; and establishes criteria for and negotiation of Assurances of Compliance with institutions engaged in PHS-conducted or supported research using animals. Conducts programs of clarification and guidance for both the Federal and non-Federal sectors with respect to the use of animals in research; and directs the development and implementation of educational and instructional programs and generates educational resource materials. Evaluates the effectiveness of PHS policies and programs for the humane care and use of laboratory animals. Serves as liaison to Presidential, Departmental, Congressional, interagency, and non-governmental Commissions and Boards established to examine issues pertaining to laboratory animal welfare in research and exercises leadership in identifying and addressing such issues.

*Additional information on the OLAW can be found at <http://grants.nih.gov/grants/olaw/olaw.htm>*

## **PRIMR**

Since its founding in 1974, Public Responsibility in Medicine and Research (PRIM&R) has been committed to the advancement of strong research programs and to the consistent application of ethical precepts in both medicine and research. Through national conferences and published reports thereon, it has addressed a broad range of issues in biomedical and behavioral research, clinical practice, ethics, and the law.

Topics addressed by PRIM&R include: The ethical and procedural issues surrounding the operation of Institutional Review Boards (IRBs) and Institutional Animal Care and Use Committees (ACUCs); educating for the responsible conduct of research; the range of problems affecting AIDS research and treatment; reproductive and other technologies and their effects on patient care; healthcare ethics committees; scientific integrity and conflicts of interest; and the general range of questions surrounding academic/industrial relations.

*Additional information on the PRIMR can be found at <http://www.primr.org/about.html>*

## **SCAW**

The Scientists' Center for Animal Welfare (SCAW) was founded in 1979. The membership consists of individuals and institutions concerned with animal welfare. This organization supports the principle that humane concern for animals should be incorporated into all areas of science; provides a forum for discussion of public accountability, public policy, and the scientist's responsibilities regarding standards of animal care and use; promotes ethical discourse on biomedical, agricultural, and wildlife experimental procedures; develops educational resource material and national guidelines on humane animal experimentation; monitors legislation concerning animal welfare; conducts workshops and surveys. The SCAW publishes a quarterly newsletter.

*Additional information on the SCAW can be found at <http://www.scaw.com/>*

## *NCI-Frederick Animal Care and Use Committee*

Public Health Service Policy, the Animal Welfare Act (as amended), and the *Guide for the Care and Use of Laboratory Animals* all require each institution to appoint an Institutional Animal Care and Use Committee (ACUC). This Committee is qualified through the experience and expertise of its members to oversee the institution's animal program, facilities and procedures.

An Assurance Letter to the Public Health Service must include the names, position titles, and credentials of the ACUC chairperson and the members. The committee shall consist of not fewer than five members, and shall include at least:

- One Doctor of Veterinary Medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program responsibility for activities involving animals at the institution;
- One practicing scientist experienced in research involving animals;
- One member whose primary concerns are in a nonscientific area (i.e., ethicist, lawyer, member of the clergy); and
- One individual who is not affiliated with the institution in any way other than as a member of the ACUC, and is not a member of the immediate family of a person who is affiliated with the institution.
- An individual who meets the requirements of more than one of the categories may fulfill more than one requirement. However, no more than three members from the same administrative unit are permitted.

### **ACUC RESPONSIBILITIES AND OVERVIEW**

The responsibilities of the NCI-Frederick ACUC are as follows:

- Review at least semi-annually the institution's program for humane care and use of animals, using the *Guide* as a basis for evaluation.
- Inspect at least semi-annually all of the institution's animal facilities (including satellite facilities) using the *Guide* as a basis for evaluation.
- Remain cognizant of animal care and use practices of NCI-Frederick investigators, remaining particularly attentive to any significant deviations from those described in approved proposals.
- Make written recommendations to the Institutional Official, NCI-Frederick, regarding any aspect of the institution's animal program, facilities, or personnel training. Apprise the Institutional Official immediately of any problems concerning use of animals for resolution, and prepare recommendations for corrective actions as may be requested.
- Review and approve, require modifications in (to secure approval) or withhold approval of those sections of applications or proposals as set forth in the PHS Policy at IV.C.
- Review and approve, require modifications in (to secure approval) or withhold approval of proposed significant changes regarding the use of animals in ongoing activities as set forth in the PHS Policy at IV.C.
- Notify investigators and the institution in writing of its decision to approve or withhold approval of those sections of applications or proposals related to the care and use of animals, or of modifications required to secure ACUC approval as set forth in the PHS Policy at IV.C.4.
- Be authorized to suspend an activity involving animals as set forth in the PHS Policy at IV.C.6.
- Review proposed methods of euthanasia other than those recommended by the American Veterinary Medical Association Panel on Euthanasia and ensure that justifiable waivers are issued in writing and filed with the ACUC Chairperson.
- Advise the Institutional Official regarding the training of professional and technical staff in animal care and use.
- Advise the Institutional Official concerning newly proposed or enacted legislation, policies, and guidelines regarding laboratory animals.

- Assist with continuance of accreditation of the animal facilities and their management practices.

The NCI-Frederick ACUC membership represents various programs involved in the use of animals. The ACUC reviews and approves, requires modifications in (to secure approval) or withholds approval for all animal research proposals conducted in NCI sponsored programs. An approved proposal is required before any animals can be procured or any study can be started. The full committee review process is as follows:

- A NCI-Frederick Animal Study Proposal form is completed and signed by the Principal Investigator and the Laboratory/Branch Chief (Scientific Director) who assure the ACUC (1) that the animals selected for the described procedures in the proposal are of an appropriate species and the minimum number required to obtain valid results; and (2) that the study has scientific merit. The same individual may not sign the form as Principal Investigator and Laboratory/Branch Chief.
- The form is sent to the ACUC Coordinator who reviews for completeness. The Coordinator then sends the form to the following individuals for pre-review:
  - Attending Veterinarian
  - Environment, Health, and Safety Program
  - IBC Coordinator [as applicable]
  - One ACUC member in a program different from that of the Principal Investigator
- The facility resource form is signed by the appropriate Animal Facility Manager who assures that there is sufficient space for housing; that there is sufficient technical expertise to conduct the study; and that any anticipated problems can be adequately handled.
- The form is returned to the ACUC Coordinator and is then brought to the ACUC for full committee review. Prior to the ACUC meeting, copies of all the study proposals are sent to each member of the committee for review.
- After a proposal has been approved, the ACUC Coordinator and/or Primary Reviewer notifies the PI in writing that the study can either start or that modifications must be submitted.

Any changes that are required to an approved Animal Study Proposal must be reviewed and approved in advance by the ACUC prior to initiation. Investigators are required to submit a modification memorandum to the ACUC Office detailing the modifications that are required for his/her study for ACUC review and approval. Failure to do so may result in the suspension of animal activities.

On an annual basis, the Principal Investigator will receive an Annual Review Notice from the ACUC Office to prompt review of the approved proposal. This form must be reviewed, completed, signed, and returned to the ACUC Office prior to the indicated deadline for ACUC review.

Every three years, the investigator will receive a Renewal Notice from the ACUC Office to prompt resubmission of his/her proposal. The investigator is required to submit a new Animal Study Proposal form prior to the indicated deadline for ACUC review and approval. Lapse in approval dates will result in the suspension of animal activities. Any animals currently in house will be transferred to a holding proposal (no data collection or experimental manipulations may be performed) until the Principal Investigator secures approval for his/her own proposal.

NOTE: Proposal and modification submission deadlines have been implemented (three weeks in advance of the regularly scheduled ACUC meeting date). Please refer to the ACUC website at <http://web.ncicrf.gov/rtp/lasp/intra/acuc/fred/main.asp> for upcoming ACUC meeting dates and applicable submission deadlines.

All NCI-Frederick Animal Study Proposal forms for studies conducted at the NCI-Frederick are kept on file with the ACUC Coordinator (LAM/LASP).

## *Guidelines for Pain/Distress Classification*

### DEFINITIONS

*Pain* is awareness of acute or chronic discomfort occurring in varying degrees of severity resulting from injury, disease or emotional distress and evidenced by biological or behavioral changes or both.

*Acute Pain* results from a traumatic, surgical or infectious event that is abrupt in onset, relatively short in duration, and generally alleviated by analgesics. Associated distress may be responsive to tranquilizers.

*Chronic Pain* results from a longstanding physical disorder or emotional distress that is usually slow in onset, has a long duration, and is generally not totally alleviated by analgesics, but frequently responds to tranquilizers combined with environmental manipulation and behavioral conditioning.

*Distress* is undesirable physical or mental stress resulting from pain, anxiety, or fear. Its acute form may be relieved by tranquilizers, whereas sustained distress requires environmental change and behavioral conditioning, and does not respond to drug therapy.

*Generalized Discomfort* is defined as any procedure that would be expected to cause more than momentary discomfort in a human and for which a human would receive pain-reducing medications including aspirin, ibuprofen, acetaminophen, lidocaine, or prescription medications.

### RISK-BENEFIT ANALYSIS

The individual performing a procedure is responsible for the prevention of pain and distress to the research animal. The level of pain and distress must be defined to properly alleviate the condition created by the procedure. Investigators should ask themselves the following questions before initiating any research project:

- Will the project yield results beneficial to animal or human health and well-being?
- Has a literature search been performed to ensure that this project is not a replication of a well-documented procedure?
- What is the rationale for involving the use of animals?
- Is the species and number of animals selected appropriate for the project?
- Is the discomfort and injury to animals limited to that which is unavoidable in the conduct of this project?

### CATEGORY 1 - Minimal, transient, or no pain or distress

These procedures are considered to produce minimal, transient, or no pain or distress when performed by competent individuals using recognized methods.

1. Administration of:
  - a. Fluid therapy
  - b. Injection of routine nonirritating substances
  - c. Oral medications
2. Blood collection (when animals are not anesthetized)
3. Gastric gavage
4. Breeding or holding colony proposals
5. Euthanasia as performed in accordance with recommendations of the AVMA Guidelines on Euthanasia [2007]
6. When using anesthesia for restraint purposes only



If the result of any of the above procedures is painful or distressful the procedure should be listed under Category 2 or Category 3 below.

#### CATEGORY 2 - Pain or distress relieved by appropriate measures

Examples of procedures that may produce pain or distress, but which are performed using appropriate and adequate anesthesia, analgesia, or tranquilization and followed with appropriate measures to alleviate pain or distress are as follows:

1. All surgical procedures or those procedures for which animals are provided with anesthesia and/or analgesia to alleviate any potential pain/distress
2. Intracardiac blood collection or injection
3. Repeat tail biopsies

#### CATEGORY 3 - Unrelieved pain or generalized discomfort

Any animal that experiences unrelieved pain or generalized discomfort without the provision of anesthetics, analgesics, or tranquilizers is placed in Category 3 under Section G of the NCI-Frederick Animal Study Proposal form. For classification purposes, a procedure causing unrelieved pain or generalized discomfort is defined as any procedure that would be expected to cause more than momentary pain or generalized discomfort in a human and for which a human would receive pain-reducing medications including aspirin, ibuprofen, acetaminophen, lidocaine, or prescription medications. To assist with the appropriate classification of animals into Category 3 (Unrelieved Pain or Generalized Discomfort) the ACUC has provided the following examples:

- *Studies that propose death as an endpoint (e.g., lethal dose studies, infectious agent models, neoplasia survival studies). Death as an endpoint is not sanctioned by the ACUC. Extenuating circumstances for which death as an endpoint is expected to require scientific justification;*
- *Pain studies that would not be possible if pain-relieving agents were administered;*
- *Psychological conditioning experiments that involve painful stimuli such as a noxious electrical shock that cannot immediately be avoided by an animal;*
- *Procedures that are known to induce a painful inflammatory response (e.g., peritonitis);*
- *Neurological studies that result in the loss of normal body function (e.g., paralysis, difficulty with ambulation, difficulty with urination/defecation, inability to obtain food or water);*
- *Studies in which treatment(s) may produce generalized discomfort as evidenced by physical (e.g., hunched posture, ruffled hair coat) or behavioral changes (e.g., decreased mobility, not eating/drinking).*

**Please note that this is not a comprehensive list of procedures that could warrant a Category 3 designation. The ACUC reviews each study on a case-by-case basis to determine if animals are undergoing procedures that require Category 3 classification. If a procedure is classified as Category 3, the investigator is required to provide the following information to the ACUC for consideration:**

- *A scientific justification as to why the appropriate use of anesthetics, analgesics, sedatives, tranquilizers, or timely euthanasia are contraindicated in the study;*
- *A description of his/her consideration of alternatives and the determination as to why alternatives are not available; and*
- *A literature search statement to include the database(s) searched, the date of the search, the period covered, and the keywords that were used.*

Additional information regarding alternatives to painful procedures and database reference searches can be found at <http://web.ncifcrf.gov/ntp/lasp/acuc/alternatives.asp>

## Reference:

Working with the ACUC: Writing an Animal Protocol. American Association for Laboratory Animal Science. April 2002.

ACUC Guidelines for Classifying Category 3 Procedures on Animals at [http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Guidelines\\_for\\_Category3.pdf](http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Guidelines_for_Category3.pdf).

## *Alternatives to Animal Models*

### **ANIMAL MODELS**

Animal models have become increasingly important as a means by which disease processes occurring in humans can be investigated. Some specific strains and stocks of animals have biological and pathologic process bearing similarities to humans, and their study can lead to a better understanding of these mechanisms. The definition of a true animal model of a particular disease, is one in which the disease in the animal is reproducible and, more important, is predictable.

In selecting an animal model, the investigator is required to establish his experimental objectives and determine that there is not an acceptable alternative to animal use. Once this is accomplished, an animal model should be selected. A good animal model should have the following attributes:

- The disease or lesion under study should be accurately reproduced and predictable
- Adequate numbers of uniform animals are available
- Small size
- Minimal genetic variability
- Environment can be standardized and controlled
- Short lifespan
- Anatomic, physiologic, and behavioral characteristics are well defined
- Readily transmissible and transplantable tumor systems are available (for cancer research)
- Easily handled

### **ALTERNATIVES TO ANIMAL MODELS**

The most widely accepted definition of "alternative model" is any technique which will reduce or eliminate the need for the use of animals in biomedical research, education, or testing, or will prevent needless suffering or pain in research animals. Six classes of alternative technique models described:

#### Physio - Chemical Techniques

The use of these techniques assist to identify human responses to chemicals and biological substances. These techniques separate complex substances and solutions into their basic elements through gas chromatography, which are then identified and measured via the use of mass spectrometry. This has been used in vitamin and drug research.

#### Computer or Mathematical Analysis

These techniques are of value when a biological effect can be represented by a known equation, computer, or mathematical analysis. These techniques can be applied as a substitute for animals, but must be validated with animal studies. The computer can manipulate, but not create, data. Until the basic data are understood in the complex physiological interactions of a living intact animal, the computer can only be used to massage data obtained from animal studies.

### Microbiological Systems

These test systems are used in toxicology and carcinogenesis studies. Many of the tests measure the capability of chemicals to induce mutating changes in a cellular DNA. The most frequent test used is the Ames Test. This system measures the ability of a chemical to cause a mutation in the bacteria, which is interpreted as the ability to induce cancer. The test has detected 80-90% of all carcinogenic chemicals that have been studied, when compared with testing results of the same compounds in animals. However, some chemicals that exhibit weak or negative reactions to this test are known to produce cancer in animals. These systems are used primarily as a screening system and must be validated with animal studies.

### Tissue/Organ Culture Preparation

These systems are used as a screening technique much the same as the Ames Test, but must have animal studies conducted to validate the results.

### Epidemiological Surveys

This system uses existing data or previously exposed species data. These surveys are useful to limit the range of investigations regarding a chemical or other substance.

### Plant Analysis

Plant substitution has had limited success by demonstrating some effects of exposure to certain substances and relate the effect to humans.

### Imaging

This system permits investigators to reduce the number of animals that may have previously been required to monitor internal in vivo function, activity, progress, etc. (i.e., metastasis).

## **ADVANTAGES**

The use of in vitro alternative techniques has several advantages:

- Reduction of the number of animals used
- Ability to obtain results more quickly
- Reduction in the cost for the tests or experiments
- Flexibility to change conditions and variables of the experiment

## **DISADVANTAGES**

There are several disadvantages to these alternative techniques:

- Basic research requires an understanding of animal metabolic responses in order to gain a fuller knowledge or understanding of the subject. With much of this unknown, the appropriate alternative cannot be selected.
- Transplant studies involving substitution of an organ, tissue, or device, can not use alternative techniques. No alternative has demonstrated the ability to accept or reject an implant.
- Surgical techniques require animal models in which to develop and perfect new techniques before use in humans.
- Pathway studies to evaluate the metabolic responses to chemicals and drugs require a living intact animal in which to test these responses.

- Idiosyncratic responses of a substance which produce an allergic or an unpredicted response cannot be tested in an alternative model. These effects do not fit any pattern or equation, which is the basis for alternative models.
- An intact animal provides a better model of the complex interaction of the physiological process in humans than does an alternative technique.

## GENERAL DISCUSSION OF THE THREE "R's"

The historical importance of animal models cannot be ignored. The use of animal models in research has contributed to the massive amount of medical knowledge on human and animal diseases. However, even with the past contributions of animal models, concerted efforts need to be made by the research community to evaluate the use of animals in research. These efforts should be directed to a more prudent use of animals and the utilization of alternative techniques. Remember the three "R's" of Russell and Burch (Replacement, Reduction and Refinement) and the fourth R of Paton (Responsibility). These "R's" are defined as replacement of animals with alternative techniques, reduction of the number of animals required for an experiment, and refinement of the experimental techniques in order to use fewer animals. The use of alternative techniques is increasing. However, until these techniques can duplicate all the interacting complex physiological factors of a living animal, animal models or humans will still be a necessary part of our biomedical research.

## ADDITIONAL INFORMATION ON ALTERNATIVES

Please refer to the ACUC website pertaining to alternatives at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/alternatives.asp>

According to the Animal Welfare Act regulations, "the ACUC shall determine that the principal investigator has considered alternatives to procedures that may cause more than slight pain or distress to the animals, and has provided a written narrative of the methods and sources used to determine that alternatives were not available." The ACUC is also responsible for ensuring that the proposed research does not unnecessarily duplicate other research. To assist the ACUC with this task, the PI is required to conduct a database reference search for all studies (regardless of species) that propose **Category 3** (unrelieved pain or generalized discomfort) procedures. The PI is required to provide the ACUC with the following information under Section G of the NCI Animal Study Proposal form for these studies: (1) a statement describing the consideration of alternatives to procedures listed for **Category 3**; (2) the PI's determination that alternatives were not available; and (3) a database reference statement to include the databases searched, the date of the search, period covered, and keywords used.

The following sites are designed to assist you with this endeavor:

NCI-Frederick Scientific Library at <http://www-library.ncifcrf.gov/veterinary.asp>

ALTWEB at <http://altweb.jhsph.edu/>

Johns Hopkins Center for Alternatives to Animal Testing at <http://caat.jhsph.edu/>

UC Davis Center for Animal Alternatives at [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm)

AWIC Alternatives and Database Searching at <http://www.nal.usda.gov/awic/pubs/IACUC/altdb.htm>

NIH Library at <http://nihlibrary.nih.gov/about/online.htm>

## *Surgery*

### GENERAL

Individuals performing minor or major procedures (survival or non-survival) should have adequate knowledge of anatomy, physiology and pharmacology in regard to the species they are using in their research project(s). All persons performing procedures should have demonstrated ability via training and experience. Good technique,

adequate facilities and support equipment; and proper pre- and post-operative care are an essential part of an animal care and use program.

## DEFINITIONS

Major surgery - includes any surgery that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy and craniotomy).

Painful procedure - any procedure that would reasonably be expected to cause more than slight or momentary pain or distress (i.e., pain in excess of that caused by injections or other minor procedures).

Non-survival surgical procedure is one in which the animal never recovers from anesthesia.

Survival surgical procedure is one in which the animal recovers from anesthesia, even if only momentarily. Survival surgery on rodents does not require a special facility but should be performed using sterile instruments, surgical gloves, and aseptic procedures.

## SURGERY AND POSTSURGICAL CARE

Requirements for surgery in laboratory animals are identified in the *Guide for the Care and Use of Laboratory Animals*, 1996 revision, pp. 11-12, 61-64, and 78-79, as reprinted below:

In general, surgical procedures are categorized as major or minor and in the laboratory setting can be further divided into survival and nonsurvival. Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation). Minor survival surgery does not expose a body cavity and causes little or no physical impairment (such as wound suturing; peripheral-vessel cannulation; such routine farm-animal procedures as castration, dehorning, and repair of prolapses; and most procedures routinely done on an "outpatient" basis in veterinary clinical practice). It is important that persons have had appropriate training to ensure that good surgical technique is practiced, that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns (Chaffee 1974; Wingfield 1979). People performing and assisting in surgical procedures in a research setting often have a wide range of educational backgrounds and might require various levels and kinds of training before they participate in surgical procedures on animals. The PHS Policy and the AWRs place responsibility with the ACUC for determining that personnel performing surgical procedures are appropriately qualified and trained in the procedures to be performed.

Aseptic technique is used to reduce microbial contamination to the lowest possible practical level (Cunliff-Beamer 1993). No procedure, piece of equipment, or germicide alone can achieve that objective (Schonholtz 1976). Aseptic technique requires the input and cooperation of everyone who enters the operating suite (Belkin 1992; McWilliams 1976). The contribution and importance of each practice varies with the procedure. Aseptic technique includes preparation of the patient, such as hair removal and disinfection of the operative site (Hofmann 1979); preparation of the surgeon, such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves (Chamberlain and Houang 1984; Pereira and others 1990; Schonholtz 1976); sterilization of instruments, supplies, and implanted materials (Kagan 1992; Ritter and Marmion 1987; Schofield 1994; Whyte 1988). In general, unless an exception is specifically justified as an essential component of the research proposal and approved by the ACUC, nonrodent aseptic surgery should be conducted only in facilities intended for that purpose. Most bacteria are carried on airborne particles or fomites, so surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic (AORN 1982; Bartley 1993). In some circumstances, it might be necessary to use an operating room for other purposes. In such cases, it is imperative that the room be returned to an appropriate level of cleanliness before its use for major survival surgery.

Presurgical planning should specify the requirements of postsurgical monitoring, care, and record-keeping, including the personnel who will perform these duties. The investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. An important component of postsurgical care is observation of the animal and intervention as required during recovery from anesthesia and surgery. The intensity of monitoring

necessary will vary with the species and the procedure and might be greater during the immediate anesthetic-recovery period than later in postoperative recovery. During the anesthetic-recovery period, the animal should be in a clean, dry area where it can be observed often by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain or discomfort during recovery from anesthesia. Additional care might be warranted, including administration of parenteral fluids for maintenance of water and electrolyte balance (FBR 1987), analgesics, and other drugs; care for surgical incisions; maintenance of appropriate medical records.

After anesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision, bandaging as appropriate, and timely removal of skin sutures, clips, or staples (UFAW 1989).

*Please refer to the ACUC Recommendations for Aseptic Technique and Post-Operative Care for Rodent Surgery at [http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Aseptic\\_Surgery\\_Postop\\_Care.pdf](http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Aseptic_Surgery_Postop_Care.pdf)*

## **ASEPTIC SURGERY**

For most rodent surgery, a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery. The facility often becomes larger and more complex as the number of animals, the size of animals, or the complexity of procedures increases, for instance, large-volume rodent procedures, the need for special restraint devices, hydraulic operating tables, and floor drains for farm-animal surgery, and procedures that require large surgical teams and support equipment and thus large space. Surgical facilities should be sufficiently separate from other areas to minimize unnecessary traffic and decrease the potential for contamination (Humphreys 1993).

For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery. The areas that support those functions should be designed to minimize traffic flow and separate the related, nonsurgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers (AORN 1982) but might also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities. The number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of postoperative wound infection (Fitzgerald 1979). Traffic in the operating room itself can be reduced by the installation of an observation window, a communication system (such as an intercom system), and judicious location of doors.

Control of contamination and ease of cleaning should be key considerations in the design of a surgical facility. The interior surfaces should be constructed of materials that are monolithic and impervious to moisture. Ventilation systems supplying filtered air at positive pressure can reduce the risk of postoperative infection (Ayscue 1986; Bartley 1993; Bourdillon 1946; Schonholtz 1976). Careful location of air supply and exhaust ducts and appropriate room-ventilation rates are also recommended to minimize contamination (Ayliffe 1991; Bartley 1993; Holton and Ridgway 1993; Humphreys 1993). To facilitate cleaning, the operating rooms should have as little fixed equipment as possible (Schonholtz 1976; UFAW 1989). Other features of the operating room to consider include surgical lights to provide adequate illumination (Ayscue 1986), sufficient electric outlets for support equipment, and gas-scavenging capability.

The surgical-support area should be designed for washing and sterilizing instruments and for storing instruments and supplies. Autoclaves are commonly placed in this area. It is often desirable to have a large sink in the animal-preparation area to facilitate cleaning of the animal and the operative site. A dressing area should be provided for personnel to change into surgical attire; a multipurpose locker room can serve this function. There should be a scrub area for surgeons, equipped with foot, knee, or electric-eye surgical sinks (Knecht and others 1981). To minimize the potential for contamination of the surgical site by aerosols generated during scrubbing, the scrub area is usually outside the operating room.

*Please refer to the ACUC Recommendations for Aseptic Technique and Post-Operative Care for Rodent Surgery at [http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Aseptic\\_Surgery\\_Postop\\_Care.pdf](http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/Aseptic_Surgery_Postop_Care.pdf)*

## MULTIPLE MAJOR SURGICAL PROCEDURES

Major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function. Multiple major survival surgical procedures on a single animal are discouraged but may be permitted if scientifically justified by the user and approved by the ACUC. For example, multiple major survival surgical procedures can be justified if they are related components of a research project, if they will conserve scarce animal resources (NRC 1990; see also footnote, p. 2 - see p. 50 of this handbook for footnote), or if they are needed for clinical reasons. If multiple major survival surgery is approved, the ACUC should pay particular attention to animal well-being through continuing evaluation of outcomes. Cost savings alone is not an adequate reason for performing multiple major survival surgical procedures (AWRs).

## *Anesthesia and Analgesia*

### REGULATIONS AND GUIDELINES

The United States Animal Welfare Act, Public Law 89-544 as amended, requires that institutional veterinarians provide guidelines regarding the use of tranquilizers, anesthetics, analgesics, and euthanasia agents as follows:

- 1) In the case of a research facility, the program of adequate veterinary care shall include the appropriate use of anesthetic, analgesic, or tranquilizing drugs, when such would be proper in the opinion of the attending veterinarian at the research facility. The use of these three classes of drugs shall be in accordance with the currently accepted veterinary medical practice as cited in appropriate professional journals or reference guides which shall produce in the individual subject animal a high level of tranquilization, anesthesia, or analgesia consistent with the proposal or design of the experiment.
- 2) It shall be incumbent upon each research facility through its Animal Care Committee and/or attending veterinarian to provide guidelines and consultation to research personnel with respect to the type and amount of tranquilizers, anesthetics, or analgesics recommended for each species of animal used by that institution.
- 3) The use of those three classes of drugs shall effectively minimize the pain and discomfort of the animals while under experimentation.

The *Guide for the Care and Use of Laboratory Animals*, 1996 revision, pages 64-65, states the following:

An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical proposals. Pain is a complex experience that typically results from stimuli that damage tissue or have the potential to damage tissue. The ability to experience and respond to pain is widespread in the animal kingdom. A painful stimulus prompts withdrawal and evasive action. Pain is a stressor and, if not relieved, can lead to unacceptable levels of stress and distress in animals. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative.

Fundamental to the relief of pain in animals is the ability to recognize its clinical signs in specific species (Hughes and Lang 1983; Soma 1987). Species vary in their response to pain (Breazile 1987; Morton and Griffiths 1985; Wright and others 1985), so criteria for assessing pain in various species differ. Some species-specific behavioral manifestations of pain or distress are used as indicators, for example, vocalization, depression or other behavioral changes, abnormal appearance or posture, and immobility (NRC 1992). It is therefore essential that personnel caring for and using animals be very familiar with species-specific (and individual) behavioral, physiologic, and biochemical indicators of well-being (Dresser 1988; Dubner 1987; Kitchen and others 1987). In general, unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals (IRAC 1985).

The selection of the most appropriate analgesic or anesthetic should reflect professional judgment as to which best meets clinical and humane requirements without compromising the scientific aspects of the research proposal.

Preoperative or intraoperative administration of analgesics might enhance postsurgical analgesia. The selection depends on many factors, such as the species and age of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the length of the operative procedure, and the safety of an agent for an animal, particularly if a physiologic deficit is induced by a surgical or other experimental procedure. Such devices as precision vaporizers and respirators increase the safety and choices of inhalation agents for use in rodents and other small species.

Some classes of drugs--such as sedatives, anxiolytics, and neuromuscular blocking agents--are not analgesic or anesthetic and thus do not relieve pain; however, they might be used in combination with appropriate analgesics and anesthetics. Neuromuscular blocking agents (e.g., pancuronium) are sometimes used to paralyze skeletal muscles during surgery in which general anesthetics have been administered (Klein 1987). When these agents are used during surgery or in any other painful procedure, many signs of anesthetic depth are eliminated because of the paralysis. However, autonomic nervous system changes (e.g., sudden changes in heart rate and blood pressure) can be indicators of pain related to an inadequate depth of anesthesia. If paralyzing agents are to be used, it is recommended that the appropriate amount of anesthetic be first defined on the basis of results of a similar procedure that used the anesthetic without a blocking agent (NRC 1992).

*For rodents, please refer to the ACUC Recommendations for Anesthetics and Perioperative Analgesia in Rodents at [http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines\\_nci.asp](http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines_nci.asp)*

*For zebrafish and frogs, please refer to the drug dosage chart at the end of the euthanasia section.*

## DEFINITIONS

Anesthesia - without sensation

Local anesthesia - loss of sensation in a limited area of the body

Regional anesthesia (nerve block) - loss of sensation to a large, though limited area of the body

Basal anesthesia - a light level of general anesthesia usually produced by preanesthetic agents

General anesthesia - state of unconsciousness characterized by absence of pain and some muscle relaxation

Surgical anesthesia - unconsciousness, accompanied by muscular relaxation to such a degree that surgery can be performed painlessly and without struggling on the part of the animal

Dissociative anesthesia - a cataleptic state resembling anesthesia accompanied by analgesia without hypnosis or depression of the central nervous system which is produced by interruption of information flow from the unconscious to the conscious parts of the brain

Analgesia - relief from pain without loss of consciousness

Tranquilization - state of behavioral change in which the animal is relaxed and unconcerned by its surroundings; the animal is often indifferent to minor pain

Neuroleptanalgesia - a state of sedation and analgesia produced by the combined use of a tranquilizer (neuroleptic) and a narcotic analgesic

Sedation - a mild degree of CNS depression in which the animal is awake but calm and free of nervousness

Narcosis - a reversible condition defined as a drug-produced state of sleep accompanied by analgesia

Hypnosis - anesthesia produced by placing the patient in a trance-like state



Acupuncture - an ancient Oriental system of therapy utilizing long fine needles that can be used to induce anesthesia

Hypothermia - lowering of body temperature, either locally or generally, to a level at which anesthesia occurs

## GENERAL PRINCIPLES

- If possible, evaluate the anesthetic technique via a literature search and in a limited trial before use in experimental procedures.
- Evaluate the health of the animal before the procedure.
- Drug selection should provide the minimal level of CNS depression necessary to provide anesthesia or analgesia.
- Consider the effect of anesthetic drugs and techniques on the experimental results and the interaction with other drugs or agents being used.
- Provide basic equipment and supplies to ensure pulmonary ventilation and to respond to emergencies.
- Monitor and maintain body temperature.
- Provide adequate postoperative care until animal is fully recovered.
- Observe all safety precautions and avoid environmental contamination.

### Factors Affecting Choice of Anesthetic and Analgesic Regimens

- Individual animal - species, age, physiologic status, etc.
- Procedure to be performed on animal - duration and surgical site
- Postoperative fate of animal
- Available equipment
- Environment - bedding material and inherent colony disease
- Personal knowledge, preference, and skill

## PREANESTHETIC CARE AND DRUGS

Water can be given ad libitum right up to the time of the procedure

Preanesthetic drugs may be given for the following reasons:

- Prevent apprehension and concomitant sympathetic stimulation
- Facilitate restraint
- Minimize side effects of anesthesia
- Reduce reflex responses
- Provide analgesia
- Adjunct to regional anesthesia; reduce the dosage of anesthetic agents

## CLASSIFICATION OF AGENTS

### Anticholinergics

*Atropine sulfate* - blocks parasympathetic impulses to cardiopulmonary system, glands and smooth muscle; often used to reduce salivation.

### Tranquilizers

These agents will produce a psychological calming of an animal but do not exert hypnotic or analgesic effects. Animals may become aroused if painful procedures are performed.

*Phenothiazine derivatives* - may lower seizure threshold; cause CNS sedation by depressing the brainstem and connections to the cerebral cortex; metabolized in liver.

*Acetylpromazine maleate* - has antiemetic, hypotensive, and hypothermic properties; often used as combination with ketamine to increase muscle relaxation; may precipitate seizures in gerbils.

*Chlorpromazine hydrochloride* - potentiates barbiturate anesthetics; IM injections in rabbits associated with severe myositis; produces teratogenic effects in rats and mice.

*Diazepam (Valium)* - Schedule IV drug with anticonvulsant properties; acts on thalamus and hypothalamus with no peripheral blocking actions; metabolized in liver.

### Analgesics

Narcotic agents and narcotic antagonists all must be handled in adherence to the Controlled Substances Act (discussed under the section on Safety).

Narcotic agents produce hypnotic and analgesic effects with resultant depression of the cardiovascular and thermoregulatory systems; attach to opiate sites in the CNS and block neurotransmitters, elevating the pain threshold; decrease the amount of other agents needed for general anesthesia by 1/3 to 1/2; most are metabolized by the liver and excreted in the bile and urine.

*Morphine* - used primarily in dogs and primates; can cause excitement in cats, horses, and food animals; duration of action of 4-6 hours; stimulates vomiting and vagal CNS centers; causes increased intraocular and intracranial pressure; relatively inexpensive.

*Meperidine (Demerol)* - preferred over morphine because of fewer side effects; used in dogs, cats, rodents, and primates; duration of action of 2-3 hours.

*Fentanyl* - potent, short acting, reversible narcotic used in Innovar-Vet; 100 times more potent than morphine; effects last 30-60 minutes.

*Buprenorphine HCL (Buprenex)* - a thebaine derivative, buprenorphine is a synthetic partial opiate agonist. It is 30 times as potent as morphine and produces a dose related analgesia. Local anesthetics may be potentiated by concomitant use of buprenorphine. Respiratory depression possible.

Narcotic antagonists (e.g., naloxone, nalorphine) are used to reverse the effects of narcotics but will not reverse the sedative or depressant effects of other drugs. Narcotic antagonists are Schedule III drugs.

Neuroleptanalgesics produce a state of sedation and analgesia produced by the combination of a tranquilizer (neuroleptic) and narcotic. The animal remains conscious and responds to certain stimuli but minor surgery can be done.

*Innovar-Vet* - narcotic analgesic fentanyl (0.4 mg/ml) plus tranquilizer droperidol (20 mg/ml); good analgesia and muscle relaxation; produces mild, atropine sensitive bradycardia; contraindicated for IM use in guinea pigs due to severe necrotic myositis at injection site; narcotic antagonists can reverse the effects of the fentanyl only.

### Non-narcotic analgesics

*Xylazine (Rompun)* - a thiazine derivative which causes sedation, muscle relaxation, and analgesia; wide margin of safety; may cause emesis via direct central stimulation; potentiates barbiturate anesthesia; may precipitate early parturition if given to animals in last month of pregnancy; yohimbine partially reverses the effects of xylazine.

*Butorphenol tartrate (Torbugesic)* - synthetic analgesic with narcotic agonist/antagonist properties. Three to five times more potent than morphine. Respiratory depressant, mild sedative. Controlled substance.

*Salicylates* - analgesic, antipyretic, and anti-inflammatory effects; aspirin is the best known salicylate; do not give to cats as can cause bone marrow depression.

*Pentazocine (Talwin)* - 1/2 as potent as morphine; duration of action averages 2 hours; produces little or no sedation; minimal effect on the cardiovascular and respiratory systems.

*Ibuprofen* – Non-narcotic, non-steroidal anti-inflammatory agent with analgesic and antipyretic properties. It is suspected to possibly reduce the platelet count as it does in humans.

*Acetaminophen* – Non-narcotic, non-steroidal anti-inflammatory agent that has similar analgesic efficacy to aspirin but has little anti-inflammatory activity.

*Ketoprofen/Carprofen* – Non-narcotic, non-steroidal anti-inflammatory agents with analgesic and antipyretic properties.

*Flunixin Meglumine* – Non-narcotic, non-steroidal anti-inflammatory agent that is a highly substituted derivative of nicotinic acid. It should be used with caution in animals with pre-existing GI ulcers, renal, hepatic or hematologic diseases.

### Anesthetics

Dissociative anesthetics - produce a state of chemical restraint and anesthesia characterized by a form of muscle rigidity and an apparent dissociation of the mind from the external environment; reflexes remain intact; tracheal intubation possible; analgesic properties questionable; produce excessive salivation that is controllable with atropine.

*Ketamine hydrochloride (Ketalar or Ketaset)* - preferred dissociative anesthetic due to a wide margin of safety; short duration and recovery time with few adverse side effects; poor muscle relaxation; contraindicated for use in animal with renal or hepatic disease; FDA approved for use in cats and nonhuman primates only. Controlled substance.

Barbiturate anesthetics - must be handled in adherence to the Controlled Substances Act (discussed under the section on Safety); will produce a state of severe depression and general anesthesia in the animal as greater doses are given; prolonged recovery with glucose, epinephrine, chloramphenicol; softwood bedding induces hepatic microsomal enzymes in rodents, reducing barbiturate sleeping time.

*Pentobarbital sodium (Nembutal)* - long acting; small safety margin.

*Thiamylal sodium (Surital)* - short acting (15 to 30 minutes).

*Tribromoethanol (Avertin)* - produces surgical anesthesia in most rodents, with good skeletal muscle relaxation and only a moderate degree of respiratory depression. It can be irritating to the peritoneum if solution is stored and decomposition results. Peritoneal adhesions may result from repeat injections.

Inhalant anesthetics - agents produce a rapid onset and recovery in an animal and have a high degree of controllability.

*Halothane (Fluothane)* - highly volatile but relatively insoluble (halothane saturated atmosphere can reach a 30% concentration); requires use of vaporizer for precise concentrations; potent cardiovascular depressant; gives fair muscle relaxation and analgesia; nonexplosive.

*Isoflurane (IsoFlo)* produces rapid induction and recovery from anesthesia and the depth of anesthesia can be altered easily and rapidly. Slightly more severe respiratory depression than halothane. Advantage is it undergoes very little biotransformation and is almost completely eliminated in exhaled air.

*Methoxyflurane (Metofane)* - highly soluble but low volatility; muscle relaxation and analgesia good; cardiovascular and respiratory depressant; nonexplosive; inhalation anesthetic of choice for rodents. Can be hazardous to humans if inhaled, i.e. renal damage.

*Nitrous oxide* - potent analgesic; useful in conjunction with halothane and methoxyflurane; nonirritating; very insoluble in blood and tissues resulting in rapid induction and recovery; nonexplosive.

### Muscle Relaxants

Neuromuscular blocking agents inhibit the transmission of nerve impulses of the neuromuscular junction resulting in skeletal muscle paralysis and profound muscular relaxation.

Depolarizing neuromuscular blocking drugs (e.g., succinylcholine) interact with and depolarize the receptor areas, causing a lack of responsiveness to acetylcholine. These agents can not be reversed.

Competitive neuromuscular blocking agents (e.g., d-tubocurarine, pancuronium) combine with the receptors and render them inaccessible to acetylcholine. These agents can be reversed.

These agents produce motor paralysis only, and do not produce either sedation or analgesia. These agents should never be used as anesthetic or analgesic agents.

## GENERAL

Various degrees of tissue damage may result from IM drug injections of several irritating anesthetics, e.g. ketamine (Gaertner, 1987, Smiler, 1990). In the case of small laboratory animals this irritation may or may not be clinically evident. In some cases, however, sufficient tissue damage can occur which leads to lameness, self-mutilation of a limb or cutaneous ulceration, thus causing some magnitude of pain and/or distress to the animal. Techniques which may be useful to minimize tissue irritation from IM drug delivery include deep rather than superficial IM delivery of the drug, use of small gauge (mouse <23 g and rat <21 g) needles, delivery of multiple small injections into several IM sites rather than injection of a large volume at one site, and dilution of the drug prior to injection with physiological saline. IM drug injections are usually delivered into either the caudal thigh muscle mass or the epaxial muscles along the spine.

To properly administer an intraperitoneal injection to a rodent, the animal should be restrained so that it is held in a head-down position with the needle inserted into the lower left abdominal quadrant. Errors in IP drug injection can be minimized by both fasting the animal for 4-8 hours prior to drug injection and by use of a 21-22g needle, rather than a small (e.g. 25 or 26g) needle which may not penetrate the subcutaneous tissue, fat and abdominal wall and thus never deliver the anesthetic to the large peritoneal surface for absorption. Scientists should also be aware of potential peritonitis caused by IP drug administration, particularly when irritating solutions are administered or bacterial contamination is introduced. Please refer to chart below.

### RECOMMENDED NEEDLE SIZES, SITES, AND VOLUMES FOR INJECTION

Species	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous	Intragastric
Rat	Scruff, back, 5-10 ml, 21 G or less	Quadriceps/posterior thigh, 0.3 ml, 23 G or less	5-10 ml, 23 G or less	Lateral tail vein, 0.5 ml, 25 G or less	5 ml

---

Mouse	Scruff, 2-3 ml, 21 G or less	Quadriceps/posterior thigh, 0.05 ml, 25 G or less	2-3 ml, 23 G or less	Lateral tail vein, 0.5 ml, 25 G or less	0.5 ml
-------	---------------------------------	---	-------------------------	---	--------

---

*Adapted from Laboratory Animals Anesthesia (P.A. Flecknell, Academic Press, 1987) and Experimental and Surgical Technique in the Rat – Second Edition (H.B. Waynforth and P.A. Flecknell, Academic Press, 1992)*

### GENERAL ADMINISTRATION NOTES

- Recommendations are based on the adult body weight of a rat [200 g] and the adult body weight of a mouse [20 g]
- These volumes should be reduced if the injected material is likely to irritate tissues OR if using an oil-based vehicle for gavaging
- Changing needles also serves to keep the injection needle from becoming dulled if passed through a stoppered vial
- For IV injections, it is recommended to use the lateral tail vein, while the mouse is enclosed within a commercial or custom made restrainer. Dilation of the vein is accomplished by warming of the tail by immersion in warm water or use of a heat lamp. Five minutes of supplemental heat may be necessary for optimal dilation. Monitor carefully to prevent the animal from being burned or overheated by the heat source. The vein is best entered in the proximal 1/3 of the tail, and a successful injection is obvious to the user [based on lack of resistance as the plunger is depressed]. If repeat injections are performed, it is recommended that user start further down the tail and to work upwards. In most cases a maximum injection volume of 0.5 ml can be safely given to an adult mouse [based on experience and veterinary observations]. Most users try to limit the injection to 0.25 ml [a standard veterinary recommendation]. For cell injections, over-concentration may lead to embolism in lung capillaries and death of the recipient. The tendency to clump is cell line dependent. Mastering of the injection technique takes practice and training sessions may be scheduled by contacting the facility manager or the LAM veterinary staff.
- For hydrodynamic gene therapy, the volumes range from 1.6 to 2.5 ml, which is given over 3-5 seconds or 6-8 seconds depending on the volume. The standard needle size used for Balb/c mice is 25 gauge and for mice with smaller tail veins [i.e., C57BL/6] a 27 gauge needle is used. Please note that it can take up to 15 seconds to administer the agent if a 27 gauge needle is used. Based on veterinary observations, the appearance of the mice post-injection is strain dependent. The Balb/c mice are not as affected by the injections as the B6 mice. The usual clinical manifestation post-injection ranges from mild immobility with the lower volume to extended periods of immobility with the higher volume. Most often, the animals are alert but have a reduced activity level [stunned appearance]. Severe dyspnea has been reported by others and if seen the animals must be humanely euthanized. For routine hydrodynamic-based transfection, anesthesia is not recommended prior to the injection due to observed gasping and delayed recovery as compared with unanesthetized animals

## *Euthanasia*

### INTRODUCTION

Euthanasia is the act of inducing painless death (Greek: eu- well + thanos- death). Criteria to be considered for a painless death are rapidly occurring unconsciousness and unconsciousness followed by cardiac or respiratory arrest. The distress experienced by people when observing euthanasia or death in any form is an emotional response dependent on the background of the observer. Kinship of people with higher animals, however distant, serves to transfer the unpleasant reaction to human death to death of animals. Such distress occurs even though the observer experiences no physical pain. This distress may be minimized by perfection of the technique of euthanasia. Although not an adequate criterion, observers may mistakenly relate any movement with consciousness and lack of movement with unconsciousness. Techniques in which animals being euthanatized exhibit little or no movement are the most aesthetically acceptable to most people.

As with other procedures applied to animals, euthanasia requires some physical control over the animal. The degree of control and kind of restraint needed will be determined by the animal species, breed, size, state of domestication, presence of painful injury or disease, degree of excitement, and method of euthanasia. Suitable control is vital to minimize pain in animals, to assure safety of the person performing euthanasia, and, frequently, to protect other animals and people.

Selection of the most appropriate method of euthanasia in any given situation is dependent on species of the animal involved, available means of animal control, skill of personnel, numbers of animals, economic factors, and other considerations. This report deals primarily with domestic animals, but the same humane considerations should be applied to all species.

Whenever an animal is in pain or distress, which cannot be relieved, it must be painlessly killed even if the experiment is not complete. The decision of whether or not to kill an animal should rest with the professional judgment of a veterinarian.

Distress may occur among personnel directly involved in performing repetitive euthanasia of diseased, injured, or unwanted animals. At the point of terminating the life of an animal, we should be prepared not only to treat the animal but also to consider the people attached to the animal. Constant exposure to or participation in euthanasia procedures can cause a psychologic state characterized by a strong sense of work dissatisfaction or alienation, which may be expressed by absenteeism, belligerence, or careless and callous handling of the animals. This is one of the principal reasons for turnover of employees directly involved with repeated performance of animal euthanasia. This should be recognized as a bona fide personnel problem related to animal euthanasia, and management measures should be instituted to decrease or eliminate the potential for this condition.

Several criteria were used in evaluating methods of euthanasia: (1) ability to produce death without causing pain; (2) time required to produce loss of consciousness; (3) time required to produce death; (4) reliability; (5) safety of personnel; (6) potential for minimizing undesirable psychologic stress on the animals; (7) nonreversibility; (8) compatibility with requirement and purpose; (9) emotional effect upon observers or operators; (10) economic feasibility; (11) compatibility with histopathologic evaluation; and (12) drug availability and abuse potential.

The NCI-Frederick Animal Care and Use Committee (ACUC) follows the AVMA Guidelines on Euthanasia [2007]. Any research proposal that involves euthanasia techniques that do not follow these guidelines must be justified to the ACUC.

For specific guidelines on rodent euthanasia, please refer to the *NCI-Frederick ACUC Guidelines for Euthanasia of Rodents* at [http://web.ncifcrf.gov/ntp/lasp/intra/acuc/fred/guidelines/Euthanasia\\_Guidelines.pdf](http://web.ncifcrf.gov/ntp/lasp/intra/acuc/fred/guidelines/Euthanasia_Guidelines.pdf)

The following methods for euthanasia of research animals, as described in the AVMA Guidelines on Euthanasia [2007], are used at the NCI-Frederick:

#### Inhalant Anesthetics

The inhalant anesthetics, primarily halothane, and methoxyflurane, have been used to euthanatize many species. With these agents, the animal is placed in a closed receptacle containing cotton or gauze soaked with the anesthetic. Vapors are inhaled until respiration ceases and death ensues. Because the liquid state of most inhalant anesthetics is a topical irritant, animals should be exposed to vapors only. Also, air or oxygen must be provided during the induction period.

Other inhalation anesthetics are seldom used for euthanasia, due to low potency (nitrous oxide), high cost (isoflurane, enflurane), or danger (cyclopropane). For example, cyclopropane is highly flammable and explosive, and requires special equipment for administration. Nitrous oxide may be used alone to produce mild analgesia, anesthesia, and death by hypoxemia. Nitrous oxide is nonflammable and nonexplosive, but will support combustion.

Advantages -- (1) The inhalant anesthetics are particularly valuable for euthanasia of birds, rodents, cats, and young dogs, i.e., animals in which venipuncture may be difficult, and (2) halothane, enflurane, isoflurane, methoxyflurane, and nitrous oxide are nonflammable and nonexplosive under ordinary environmental conditions.

Disadvantages -- (1) Struggling and anxiety may occur during induction of anesthesia because anesthetic vapors are irritating and induce excitement; (2) ether is flammable and explosive and should not be used near an open flame or other ignition sources (may not be used for euthanasia at the NCI-Frederick); (3) personnel and other animals can be injured by exposure to these agents; and (4) halothane, methoxyflurane, nitrous oxide, enflurane, and isoflurane are relatively expensive.

Recommendations -- Under certain circumstances, halothane, isoflurane, enflurane, methoxyflurane, and nitrous oxide administered by inhalation are acceptable for euthanasia of small animals (i.e., birds, rodents, cats, and young dogs). Although acceptable, these agents generally are not used in larger animals because of their cost and difficulty of administration. In emergency situations, halothane, enflurane, or isoflurane may be administered to large animals.

### Carbon Dioxide

Room air contains 0.04% carbon dioxide (CO<sub>2</sub>). Pure CO<sub>2</sub> is heavier than air and nearly odorless. Inhalation of CO<sub>2</sub> in concentrations of 7.5% increases the pain threshold, and higher concentrations of CO<sub>2</sub> have a rapid anesthetic effect.

Inhalation of 60% CO<sub>2</sub>, results in loss of consciousness within 45 seconds, and respiratory arrest within 5 minutes. Carbon dioxide has been used to euthanize groups of small laboratory animals, including mice, rats, guinea pigs, chickens, and rabbits, and for humane slaughter of swine for human consumption. According to Croft, animals do not detect the CO<sub>2</sub> immediately, and its depressant action takes place almost unnoticed.

Leake and Waters reported the experimental use of CO<sub>2</sub> as an anesthetic agent in the dog. Thirty percent to 40% CO<sub>2</sub> in oxygen induced anesthesia within 1 to 2 minutes, usually without struggling, retching, or vomiting. The combination of 40% CO<sub>2</sub> and approximately 3% CO has been used experimentally for euthanasia of dogs by Carding. Carbon dioxide has been used in specially designed chambers to euthanize cats and other small laboratory animals.

Studies in day-old chickens have shown that CO<sub>2</sub> is an effective euthanizing agent. Inhalation of CO<sub>2</sub> caused little distress to the birds, suppressing nervous activity and inducing death rather quickly. Because respiration begins during embryonic development, the unhatched chickens' environment may normally have a CO<sub>2</sub> concentration as high as 14%. Thus, CO<sub>2</sub> concentration for euthanasia for baby chickens and other neonates should be especially high. A CO<sub>2</sub> concentration of 60% to 70% with a 5-minute exposure time appears to be optimal.

Advantages -- (1) The rapid depressant and anesthetic effects of CO<sub>2</sub> are well established; (2) Carbon dioxide may be purchased in compressed gas cylinders; (3) CO<sub>2</sub> is inexpensive, nonflammable, and nonexplosive, and presents minimal hazard to personnel when used with properly designed equipment; (4) CO<sub>2</sub> does not result in accumulation of tissue residues in food producing animals; and (5) CO<sub>2</sub> euthanasia does not distort murine cholinergic markers or corticosterone concentrations.

Disadvantages -- (1) Because CO<sub>2</sub> is heavier than air, incomplete filling of a chamber may permit a tall or climbing animal to avoid exposure and survive; (2) some species, such as fish and burrowing, diving mammals, and neonatal rodents, may have extraordinary tolerance to CO<sub>2</sub>; (3) reptile and amphibians may breathe too slowly for the use of CO<sub>2</sub>; (4) euthanasia by exposure to CO<sub>2</sub> may take longer than euthanasia by other means; (5) induction of loss of consciousness at lower concentrations (<80%) may produce pulmonary and upper respiratory tract lesions; and (6) high concentrations of CO<sub>2</sub> may be distressful to some animals.

Recommendations -- Carbon dioxide is acceptable for euthanasia in appropriate species. Compressed CO<sub>2</sub> gas in cylinders is the only recommended source of CO<sub>2</sub> because the inflow to the chamber can be regulated precisely. CO<sub>2</sub> generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable. Species should be separated and chambers should not be overcrowded. With an animal in the chamber, an optimal flow rate should displace at least 20% of the chamber volume per minute. Loss of consciousness may be induced more rapidly by exposing animals to a CO<sub>2</sub> concentration of 70% or more by prefilling the chamber for species in which this has not been shown to cause distress. Gas flow should be

maintained for at least one minute after apparent clinical death. It is important to verify that an animal is dead before removing it from the chamber. If an animal is not dead, CO<sub>2</sub> narcosis must be followed with another method of euthanasia.

### Barbituric Acid Derivatives

Barbiturates depress the central nervous system in descending order, beginning with the cerebral cortex. Within seconds of intravenous administration, unconsciousness is induced and it progresses to deep anesthesia. Apnea occurs due to depression of the respiratory center, and cardiac arrest quickly follows. Several barbiturates are acceptable, but pentobarbital sodium most commonly is used for euthanasia.

Advantages -- (1) A primary advantage of barbiturates is speed of action. This effect depends on the dose, concentration, and rate of injection; (2) the barbiturates induce euthanasia smoothly, with minimal discomfort to the animal; (3) barbiturates are less expensive than many other euthanasia agents.

Disadvantages -- (1) Intravenous injection is necessary for best results, necessitating trained personnel; (2) each animal must be restrained; (3) current federal drug regulations require strict accounting for the barbiturates and, by necessity, these must be used under the supervision of personnel registered with the U.S. Drug Enforcement Agency; and (4) an aesthetically objectionable terminal gasp may occur in the unconscious animal.

Recommendations -- The advantages of using barbiturates for euthanasia in small animals far outweigh the disadvantages. The intravenous injection of a barbituric acid derivative is the preferred method for euthanasia of dogs, cats, and other small animals. Intraperitoneal injection may be used in situations when an intravenous injection would be distressful or even dangerous. Intracardiac injections must only be used if the animal is heavily sedated, unconscious, or anesthetized.

### Cervical Dislocation

Cervical dislocation is used to euthanize poultry, mice, and immature rats and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.

Advantages -- (1) Cervical dislocation is a technique that may induce rapid loss of consciousness; (2) does not chemically contaminate tissues; and (3) it is rapidly accomplished.

Disadvantages -- (1) May be aesthetically displeasing to personnel; (2) it requires mastering technical skills to ensure loss of consciousness is rapidly induced; and (3) its use is limited to poultry, mice, and immature rats and rabbits.

Recommendation -- Manual cervical dislocation is a humane technique for euthanasia of poultry, other small birds, mice, rats weighing <200 g, and rabbits weighing less than <1 kg when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be sedated or anesthetized prior to cervical dislocation. The need for technical competency is greater in heavy rats and rabbits, in which large muscle mass in the cervical region makes manual cervical dislocation physically more difficult. In research settings, this technique should be used only when scientifically justified by the user and approved by the ACUC.

### Decapitation with Guillotine

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate that this activity does not infer the ability to perceive pain, and in fact conclude that loss of consciousness develops rapidly. Guillotines that are designed to accomplish decapitation in adult rodents and small rabbits in a uniformly



instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose.

Advantages -- (1) Decapitation is a technique that appears to induce rapid loss of consciousness; (2) it does not chemically contaminate tissues; and (3) it is rapidly accomplished.

Disadvantages -- (1) Handling and restraining required to perform this technique may be distressful to animals; (2) the interpretation of the presence of electrical activity in the brain following decapitation has created controversy and its importance may still be open to debate; (3) personnel performing this technique should recognize the inherent danger of the guillotine and take adequate precautions to prevent personal injury; and (4) decapitation may be aesthetically displeasing to personnel performing or observing the technique.

Recommendation -- This technique is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental designed and approved by the ACUC. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress form handling, minimized the chance of injury to personnel, and improves positioning of the animal in the guillotine. Decapitation of amphibians, fish, and reptiles is addressed elsewhere in this report. Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so.

Exsanguination

Exsanguination can be used to ensure death subsequent to stunning, or in otherwise unconscious animals. Because anxiety is associated with extreme hypovolemia, exsanguinations must not be used as a sole means of euthanasia. Animals may be exsanguinated to obtain blood products, but only when they are sedated, stunned, or anesthetized.

**RODENT FETUS ANESTHESIA AND EUTHANASIA**

Acceptable methods for euthanasia of neonatal mice and rats are described below. In all cases, personnel performing the euthanasia must be fully trained in the appropriate methods. Please consult veterinary staff with additional questions or concerns.

AGE	RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS <i>(In order of recommended use)</i>	NOTES
Day 1-6	Decapitation with sharp surgical scissors	Use is at the discretion of the individual performing euthanasia. Individual must demonstrate proficiency at this technique
	Anesthesia by hypothermia & decapitation	Rodent is placed in specimen cup (not petri dish) and submerged into an ice slurry for 20 minutes. <i>This method is used to decrease or terminate movement prior to decapitation, reduce residual nervous activity pre- and post-decapitation and reduce bleeding post-decapitation.</i>
	CO <sub>2</sub> followed by decapitation with surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation

AGE	RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS (In order of recommended use)	NOTES
	CO <sub>2</sub> alone (not recommended as sole method)	Not a recommended method as it requires that animal remain in the CO <sub>2</sub> chamber >20 min. after cessation of all movement is observed; after removal from CO <sub>2</sub> chamber an additional >15 min. of observation is required to ensure death has occurred. A recommended method for use prior to decapitation with sharp surgical scissors.
Day 7-14	CO <sub>2</sub> followed by decapitation with sharp surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation
	CO <sub>2</sub> alone	Requires that animal remain in the CO <sub>2</sub> chamber >10 min. after cessation of all movement is observed; after removal from CO <sub>2</sub> chamber an additional >15 min. of observation is required to ensure death has occurred.

## EUTHANASIA OF AMPHIBIANS AND FISH

Euthanasia of ectothermic animals must take into account differences in their metabolism, respiration, and tolerance to cerebral hypoxia. In addition, it is often more difficult to ascertain when an animal is dead. Some unique aspects of euthanasia of amphibians, fishes, and reptiles have been described.

Tricaine methane sulfonate (TMS, MS-222) may be administered by various routes to euthanize. For fish and amphibians, this chemical may be placed in water. MS-222 is acidic and in concentrations  $\geq 500$  mg/L should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5. MS-222 may also be injected into lymph spaces and pleuroperitoneal cavities. These are effective but expensive means of euthanasia. The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed. For euthanasia, a concentration  $\geq 250$  mg/L is recommended and fish should be left in this solution for at least 10 minutes following cessation of opercular movement.

Benzocaine hydrochloride, a compound similar to TMS, may be used as a bath or in a recirculation system for euthanasia of fish or amphibians. Benzocaine is not water-soluble and therefore is prepared as a stock solution (100 g/L), using acetone or ethanol, which may be irritating to fish tissues. In contrast, benzocaine hydrochloride is water-soluble and can be used directly for anesthesia or euthanasia. A concentration  $\geq 250$  mg/L can be used for euthanasia.

Amphibians, reptiles, and fish may be euthanized with CO<sub>2</sub>. Loss of consciousness develops rapidly but exposure times required for euthanasia are prolonged.

Chilling frogs in ice slurry for 20 minutes followed by decapitation is acceptable. Another acceptable method is anesthetizing with 0.1% tricaine-methane sulfonate solution for approximately 30 minutes followed by decapitation. Decapitation with heavy shears or a guillotine is effective for some species that have appropriate anatomic features. It has been assumed that stopping blood supply to the brain by decapitation causes rapid loss of consciousness.

It has been suggested that, when using physical methods of euthanasia in ectothermic species, cooling to 4 C will decrease metabolism and facilitate handling, but there is no evidence that whole body cooling reduces pain or is

clinically efficacious. Local cooling in frogs does reduce nociception, and this may be partly opioid mediated. Quick freezing (-70 C) of deeply anesthetized animals is acceptable.

Sodium pentobarbital (60 to 100 mg/kg of body weight) can be administered intravenously, intraabdominally, or intrapleuroperitoneally in most ectothermic animals, depending on anatomic features. Subcutaneous lymph spaces may also be used in frogs and toads. Time to effect may be variable, with death occurring in up to 30 minutes. Barbiturates other than pentobarbital can cause pain on injection.

### *African Clawed Frog*

Xenopus sp

#### Anesthesia

Tricaine (MS 222) - Immerse in water with agent added:  
500-2000 mg/L bath (buffer to pH of 7.0 to 7.5 with NaH CO<sub>3</sub>)  
Induction in 15-30 minutes; maintain by moist cloth contact with MS 222 solution.  
Recovery - keep at 22-26°C; takes 3-6 hours; keep moist.

#### Euthanasia

- Chilling at 4°C for 20 minutes to provide sedation - euthanize by decapitation with sharp scissors.
- Overdose of anesthesia.
- Anesthesia followed by decapitation.
- Anesthesia followed by deep freeze at -70 °C.

### *Zebrafish*

#### Anesthesia

Tricaine (MS 222) - Fish are induced rapidly following immersion in a solution containing MS-222 (100-200 mg/L - buffer to pH of 7.0 to 7.5 with NaH CO<sub>3</sub>)<sup>12,19</sup> and are recovered by returning them to fresh, well-aerated water. Because most procedures performed on zebrafish are very rapid, the need for a maintenance phase of anesthesia is usually not necessary. Maintenance anesthesia doses would be lower (50-100 mg/L)<sup>19</sup>. During induction, spontaneous ventilation should be monitored closely and can be used as an indicator to the depth of anesthesia.

#### Euthanasia

- For euthanasia, a concentration  $\geq 250$  mg/L is recommended and fish should be left in this solution for at
- least 10 minutes following cessation of opercular movement.
- Overdose of anesthesia.
- Anesthesia followed by decapitation.
- Anesthesia followed by deep freeze at -70 °C.
- Anesthesia followed by fixative for necropsy

## DRUG ENFORCEMENT ADMINISTRATION

The use of narcotics and other drugs of potential abuse is controlled by the Drug Enforcement Administration (DEA) of the U.S. Department of Justice. The present laws under which DEA operates are the Controlled Substances Act and the Controlled Substances Import and Export Act. These acts regulate the manufacture, distribution, and use of potentially abused drugs and are found in 21 CFR 1300 to 1316.

Drugs that come under the jurisdiction of the Controlled Substances Act are divided into five schedules:

Schedule I - drugs that have no accepted medical usage in the United States and have high abuse potential. They can be obtained for research purposes from DEA. Examples include heroin, marijuana, LSD, peyote, dihydromorphine, methaqualone, and dimethylamphetamine.

Schedule II - drugs with high abuse potential that produce severe psychic or physical dependence, but can have medical application. Examples include pentobarbital, codeine, morphine, opium, meperidine (Demerol), oxycodone (Percodan), etorphine, amphetamines and methamphetamines.

Schedule III - drugs with abuse potential less than those in Schedules I and II, and include compounds containing limited quantities of certain narcotic and non-narcotic drugs. Examples include paregoric, narcotic antagonists, and barbiturates not found in other schedules. Ketamine Hydrochloride is now a controlled substance. As of February 27, 1991, all anabolic steroids have been listed under Schedule III. The term "anabolic steroid" means any drug or hormonal substance chemically and pharmacologically related in testosterone that promotes muscle growth (other than estrogens, progestins, and corticosteroids).

Schedule IV - drugs with limited psychic or physical dependence hazards. Examples include phenobarbital, chloral hydrate, diazepam (Valium), paraldehyde, pentazocine (Talwin), butorphanol tartrate.

Schedule V - drugs with low abuse potential; consist primarily of preparations containing limited quantities of certain narcotic drugs, generally for antitussive and antidiarrheal purposes and available without a prescription (Example – buprenorphine hydrochloride).

To insure that drug supplies are adequately protected drugs shall be purchased, stored, and inventoried in accordance with NCI-Frederick Policy and Procedure 606 - Controlled Substances. A synopsis of the requirements follows:

- Designate a responsible individual to act as Drug Control Officer
- Maintain a written, current inventory on Controlled Substances Logbook (obtained from Safety)
- Record in the logbook all controlled substances used on animals
- Record in the logbook any drugs that are wasted or disposed of (call Safety to dispose of all expired drugs)
- Make sure all drugs, needles, and syringes are locked in a secure cabinet and are safe when unattended
- Keep unauthorized people out of the lab areas

The ACUC provides the following guidance related to drug dosing as they may apply to a research study: Calculations for Making Drug Preparations, Proper Drug Concentrations for Compound Delivery to an Animal, and Cell Preparations at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/ACUC39.00Dosing.pdf> and Species Dosage Conversion Factors at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/ACUC42EquivSurfAreaDosageConversion.pdf>

## *Animal Health and Disease*

### LABORATORY ANIMAL MEDICINE PROGRAM

The responsibilities of the Laboratory Animal Medicine (LAM) program include:

- Directing animal disease control programs
- Providing consultation to investigators to assure appropriate animal model selection and humane utilization of animals
- Providing of a clinical veterinary medicine program, including clinical diagnosis and therapy and close coordination with the Animal Health Diagnostic Laboratory (AHDL) in monitoring programs and receiving and quarantine procedures
- Providing a comprehensive preventive medicine program to prevent disease in laboratory animals and to prevent the spread of zoonotic diseases
- Providing consultation regarding the selection and use of anesthetic, analgesic, and tranquilizing drugs
- Providing consultation and assistance with surgical procedures and preoperative and postoperative care
- Formulating policies and standards for animal purchase and receipt
- Evaluating research facilities for design, animal housing systems, environmental control, and general operation so as to minimize research complications due to environmental variation or animal disease
- Establishing procedures to assure compliance with federal policy, regulations, and legislation governing the ethical use of animals in biomedical research
- Coordinating the response of the Animal Health Diagnostic Laboratory (AHDL), the Pathology/Histotechnology Laboratory (PHL), and the Animal Holding and Technical Support Services to animal disease problems and interacting directly with appropriate NCI/NIH officials to inform and obtain concurrence on proposed courses of action
- Providing assistance to the NCI-Frederick Animal Production Area [CRL] program
- Reviewing of all animal use and research proposals at the NCI-Frederick
- Providing mandatory training for all new and existing LAMP employees on the care and use of animals used in research
- The Attending Veterinarian serves as the Manager of the Receiving and Quarantine Program

### ZOONOSES

A zoonosis is an infection or an infectious disease transmissible under natural conditions between animals and humans (plural: zoonoses).

Animals can serve as natural reservoirs for infectious diseases, are sources of allergens, and are often used in research studies involving pathogenic microorganisms. There are over 150 diseases of vertebrate animals that are transmissible to humans under natural conditions. Individuals working with animals have a higher risk for contracting one of these diseases than the general population. These biological hazards can affect not only laboratory personnel, but also valuable research animals and breeding stock. Infectious diseases that remain unnoticed can introduce unwanted variables into research projects and invalidate results. Information on specific diseases can be found in Control of Communicable Diseases in Man, 18<sup>th</sup> ed. (Heymann, D.L., editor; American Public Health Association).

Animal bites are a constant hazard for individuals working with laboratory animals. In addition to trauma and the possibility of localized infection, many diseases can be transmitted to humans via animal bites. These include rabies, tetanus, lymphocytic choriomeningitis, cat scratch fever, rat bite fever, tularemia, and herpes B (Herpes simiae). Animal bite wounds should always be cleaned by copious local irrigation with soap and water. Tetanus toxoid and prophylactic antibiotics may be indicated. As with any occupational injury, inform your supervisor and report to Occupational Health Services for the provision of appropriate medical treatment.

Laboratory animals are sources of potent allergens to sensitized persons. Laboratory Animal Allergy is now a widely recognized clinical syndrome and hypersensitivity to animal allergens is a significant occupational health hazard for laboratory workers. Substances in the urine, skin, hair, and saliva of many common laboratory species

can cause symptoms ranging from mild rhinitis to debilitating asthma in 10 to 33% of exposed workers. Animal allergies pose a serious threat to research institutions that stand to lose highly trained individuals.

Protection of laboratory personnel from zoonoses and allergens:

1. Personal hygiene - always wash hands after handling animals and infectious materials
2. Wear protective clothing, such as face mask, shoe covers, and lab coat or coveralls; wear appropriate gloves when handling animals; do not wear this protective clothing outside the facility
3. Restrict eating, drinking, and smoking to designated areas
4. Maintain frozen serum samples on all individuals working with animals
5. Observe strict quarantine procedures for sick animals or for new animals entering facilities
6. Testing of biological materials such as tumor cell lines, for the presence of infectious agents (i.e., murine antibody tests - MAP/RAP)
7. Minimize or contain all dust and aerosol producing activities (e.g., changing bedding, cleaning animal holding areas, and necropsies)
8. Use cage top filters
9. Careful disposal of animal wastes, bedding, and dead animals (e.g., incineration)
10. Careful handling of sharps, needles, and syringes that have had contact with animal tissue
11. Efficient air handling system
12. Restrict access to animal facilities

## SIGNS OF ANIMAL DISEASE

Observe your animals on a daily basis [more often if necessary] and maintain an awareness of conditions that may indicate overt or unapparent disease. Any of the following conditions could be caused by spontaneous disease, experimental study, or traumatic injury:

Debilitated	Tumor(s)	Paralysis	Diarrhea
Dehydrated	Abscess	Ataxia	Rectal Prolapse
Emaciated	Lameness	Seizures	Blood or exudates in cage
Listless	Dermatitis	Coughing/Sneezing	Skin ulceration
Comatose	Swelling	Discharges from body orifices	Hypothermia
Dyspnea	Open wound	Rough hair coat	Perianal soiling
Alopecia	Circling	Growth retardation	
Scratching	Head tilt		

Observation of any of the above conditions should be followed up by a diagnosis, prognosis and treatment regimen after consulting the LAM veterinary staff. Animals may be euthanized humanely for disease control or to alleviate pain or distress.

## ANIMAL PROCUREMENT

### Approved sources

Most of the rodents used at the NCI-Frederick are produced at the Animal Production Area (operated by Charles River Laboratories). This facility breeds mice and rats.

Other sources of animals include those known to be free of pathogens that would be a hazard to the established NCI-Frederick animal holding colonies. Animals are generally procured from the following sources:

*Jackson Laboratories (Bar Harbor Maine) - mice*  
*Charles River Breeding Laboratories (Wilmington MA, Portage MI, Raleigh NC, and Kingston NY) - mice and rats*  
*Taconic (Germantown NY) - mice and rats*

Approved source animals are permitted to be directly shipped into the following bypass facilities: 538, 539-1CB, 550, 567 limited access, 1023, 1036, 1037, 1047 and 1049. For all other Frederick facilities, animals are required to be received through the Receiving and Quarantine facility.

#### Nonapproved sources

If the required animal/strain/stock is not available from the clean sources noted above, animals may be obtained from nonstandard sources. Freedom from pathogens must be shown by thorough health monitoring which includes clinical evaluation, serology, bacteriology, parasitology, and histopathology. Please contact the Receiving and Quarantine area for additional details on importing animals from nonapproved sources.

Extra animals should be requested by investigators for each incoming shipment from these nonstandard sources to permit appropriate health testing and to insure that the numbers of animals required for the planned research will not be compromised. In addition to testing study mice directly, sentinel health monitors from the Animal Production Area are used to monitor all non-standard source animals while they are in quarantine. The sentinel animals will be exposed to the incoming animals for at least six weeks prior to testing.

Animals that do not meet NCI-Frederick health requirements to be free from infectious disease and parasites may be housed at an off-site location or rederived.

## **QUARANTINE, STABILIZATION, AND ISOLATION**

#### Quarantine of rodents

Rats and mice from the NCI-Frederick Animal Production Area are generally not quarantined unless specifically requested by the investigator or facility manager. These animals are delivered directly to the animal holding facilities.

Rodents from outside sources are quarantined at the NCI-Frederick quarantine facility (Building 429) for non-bypass animal facilities. Daily health examinations are conducted on all animals by animal technicians and/or the Supervisor/Manager of the Receiving and Quarantine Facility during quarantine. The LAM veterinary staff is responsible for initiation of therapy if necessary, or for other disposition as required. Each shipment of rats and mice coming from approved sources is evaluated as follows:

#### a) *Mice and rats*

Quarantine period is dependent on the age and source of the animals received.

Serologic testing is conducted based on shipment size and source. Generally, 40% of the animals are tested but all mice in smaller shipments may be tested. For immunocompetent animals, serum samples for viral antibody are collected on arrival, 21 days, and 42 days after receipt in animals six weeks of age or older. Animals less than six weeks of age are held until they reach that age before serum samples are collected. Immunocompromised animals are not bled for serology. Sentinel animals are bled at the end of the six week quarantine period.

Anal tape examinations for endoparasites and hair samples for ectoparasites are conducted on 20-40% of the animals. All animals are tested for smaller shipments. Samples are collected on arrival, 14 days and 42 days post-receipt. Fecal samples from each cage are cultured for bacterial pathogens and examined for endoparasites.

#### Stabilization

Rodents are transferred to the appropriate animal holding colonies following the initial quarantine period. At that time, they are held for a period of time to allow for acclimation to their new environment.

#### Isolation for Sick Animals

The Laboratory Animal Medicine program is notified whenever a sick animal is found in any of the rodent facilities. No facility exists for the isolation of sick rodents. Sick animals are generally treated (depending on the nature of illness) or removed for necropsy. Animal rooms or an entire facility may be quarantined if animals become infected with a pathogenic organism.

*For additional information, please visit the Receiving and Quarantine website at <http://web.ncifcrf.gov/rtp/lasp/intra/randq/default.asp>*

## **REDERIVATION OF MICE**

The NCI-Frederick Receiving and Quarantine [Building 429] Animal Facility offers the opportunity to import mice for the purpose of removing undesirable organisms through rederivation. Mice of unknown pathogenic background, or those with a known undesirable pathogen, may be imported and kept in special PIV racks for the purpose of removing these undesirable organisms through Caesarian Rederivation or Embryo Transfer Rederivation. After rederivation and testing, mice may then be released and shipped to clean facilities.

*For additional information, please visit the Receiving and Quarantine website at <http://web.ncifcrf.gov/rtp/lasp/intra/randq/default.asp>*

## **SEPARATION BY SPECIES, SOURCE, AND HEALTH STATUS**

Physical separation of animals by species is recommended to prevent interspecies disease transmission, and to eliminate anxiety and possible physiologic and behavioral changes due to interspecies conflict (*Guide for the Care and Use of Laboratory Animals*). On occasion, different rodent species are housed in the same animal holding rooms at several locations at the NCI-Frederick. Mixed species include rats and mice. In most cases, these species are being used on similar studies (e.g., AIDS research or feeding of carcinogens) and/or are from the same source with a similar health status.

## **SURVEILLANCE, DIAGNOSIS, TREATMENT, AND CONTROL OF ANIMAL DISEASES PROGRAM**

The animal health program is implemented through close cooperation and interaction among personnel from the animal facilities, Laboratory Animal Medicine, the Animal Health Diagnostic Laboratory (AHDL), the Animal Molecular Diagnostic Laboratory, the Pathology/Histotechnology Laboratory, and the Animal Production Area. All animals are observed daily by the animal caretakers for signs of illness or abnormal behavior. These examinations are supplemented by observations by the managers of the animal holding colonies, investigators, research technicians, and the LAM veterinary staff.

Any animal health problem or potential health problem is immediately reported to the appropriate animal facility manager. The facility manager then notifies the attending veterinarian.

The Laboratory Animal Medicine veterinary staff provide clinical diagnoses, directs animal treatments, directs disposition of affected animals, and proposes courses of action. Some treatments require direct veterinary attention. Others treatments are done by animal technicians under the direction of the Head, Laboratory Animal Medicine.

The health of the NCI-Frederick animal holding colonies is evaluated and maintained by:

- Daily health examinations
- Examining and testing all cases of spontaneous clinical illness or abnormalities
- Testing sentinel animals on monthly and quarterly schedules
- Quarterly random colony testing
- Obtaining animals primarily from the NCI-Frederick Animal Production Area
- Quarantine of all animals from outside sources



Laboratory results and necropsy/histopathology reports for animals from the rodent colonies are maintained on file at the Animal Health Diagnostic Laboratory and in the individual animal facilities. The results can also be found online at <http://web.ncifcrf.gov/rtp/lasp/intra/ahdl/reports.asp>

## ANIMAL HEALTH PROGRAMS

The Animal Health Diagnostic Laboratory and Laboratory Animal Medicine conduct constant disease monitoring programs for the animal holding colonies, the receiving and quarantine area, and the Animal Production Area. The program is conducted on a specific time schedule and consists of:

### Sentinel Animal Program

- a) Sentinel animals are obtained from the Animal Production Area and are free of specific pathogens.
- b) They are placed with animals of the same species to aid in the evaluation of animal health. Sentinel animals are used to monitor mouse and rat colonies. The following strains of animals are used as sentinels in the animal holding colonies:

*mice – NIH SWISS or Cr:NIH(s)*

*rats - F344/NCr*

- c) Sentinel animals are 4 weeks of age at the time of entry into the sentinel program. Sentinel animals are held 3 animals/cage/4 racks/species/room. They are housed in cages containing bedding used previously to house animals from every cage within the room. The sentinel animals are kept on the bottom shelves of racks without filter tops (facility dependent) to increase exposure to room aerosols.
- d) Sentinel animals are held in each room at least one month prior to submission to the AHDL. Sentinel animals are tested monthly, and quarterly.
- e) Semiannually, complete necropsies are done on sentinel animals and tissues are taken for histopathologic examination. Quarterly, samples are also collected for serologic, parasitologic, and bacteriologic evaluation. A comprehensive screen and intermediate screen are each performed semi-annually, alternating between the two screen types.
- f) Quarterly, random noninvasive sampling for parasites is done in each animal holding facility. These tests are in addition to the sentinel monitoring program. The following samples are collected:

*mice and rats - tapes, hair, and feces*

- g) Monthly samples of animal feed, bedding, drinking water, RODAC plates, spore strips, and environmental swabs are submitted to the AHDL to monitor the effectiveness of autoclaving and sanitation procedures.
- h) Animals, serum samples, environmental samples, etc. may be submitted for clinical evaluation at the request of an investigator, facility manager, or the veterinarian in charge whenever there is a change in the animal facility health status. Submissions are tested for the presence of murine viral and mycoplasma antibody, pathogenic bacteria, ectoparasites, or endoparasites.

### Animal Production Area

- a) Monthly and quarterly submission of approximately 10 to 20 animals (retired breeders and weanlings) of each species from each building. These animals act as sentinels for the production colonies.
- b) Animals, serum samples, environmental samples, etc. may be submitted for clinical evaluation at the request of an investigator, facility manager, or the veterinarian in charge whenever there is a change in the

animal facility health status. Submissions are tested for the presence of murine viral and mycoplasma antibody, pathogenic bacteria, ectoparasites, or endoparasites.

## DIAGNOSTIC RESOURCES

The Animal Health Diagnostic Laboratory (AHDL), located in Building 429 (301-846-1134) and the Animal Molecular Laboratory (AMDL), located in Building 538 (301-846-1053) are full-service facilities with independent or collaborative capabilities for diagnosis and interpretation of the health status of laboratory animals and animal facility sanitation efforts. There is a cohesive interaction between Laboratory Animal Medicine, Laboratory Animal Holding and Technical Support, and the AHDL, providing maximal diagnostic and veterinary medical care to support biomedical research at the NCI-Frederick. The AHDL maintains extensive records that summarize and interpret all results from animal health and environmental testing.

Please refer to the Testing Requirements for Biological Materials Proposed for Use in NCI-Frederick Animal Study Proposals at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/ACUC41TestingBiologicalMatl.pdf>

The AHDL conducts and/or coordinates testing for the following diagnostic areas:

Viral serology - The AHDL has the capability to detect viral and mycoplasmal antibody utilizing traditional and modern techniques including MFIA, ELISA, and IFA tests. The AHDL utilizes an independent commercial laboratory for conformation of unusual test results.

Bacteriology - Several culture methods are employed to identify pathogenic microorganisms. The laboratory has the capability to provide more sophisticated culture techniques for clinical cases, including anaerobic and special gas environments, phase microscopy, and serological typing.

Parasitology - Direct examination of animals, microscopic examination of hair, anal tapes, fecal samples (flotation), and intestinal scrapings are methods employed to detect the presence of ecto- and endoparasites.

Mouse Antibody Production and Rat Antibody Production Testing (MAP/RAP)/Molecular Testing of Biological Materials (MTBM-M/R) - All biological materials (e.g., tumor cell lines, cell cultures, ascitic fluids, and tissues) must be tested to make certain that there is no murine viral contamination before they may be used in laboratory animals. For the MAP/RAP Test, virus antibody free animals are inoculated with the test material. Antibody levels are measured three weeks later with standard serological methods. Lactic Dehydrogenase (LDH) virus contamination is detected by measuring elevated LDH levels after inoculation of virus free animals. For the MTBM-M/R, samples are submitted to an outside laboratory, which employs molecular techniques to examine for the presence of murine viral contaminants. The AHDL will make the determination of which assay is best utilized, based upon the nature/type of material and passage history. A copy of the MAP/RAP or MTBM-M/R test results must accompany NCI Animal Study Proposal forms.

Molecular Diagnostics - the AMDL has an number of PCR-based assays for the detection of pathogenic organisms.

Genetic monitoring - The AMDL supports the Mouse Models of Human Cancer Consortium Repository (MMHCC) with molecular based genotyping services.

Gut flora - Gnotobiotic animals, housed at Animal Production Area and other off site locations, are monitored for bacterial and fungal contaminants as well as for the presence of the Charles River Amended Schaedler Flora using standard aerobic and anaerobic culture methods.

Histopathology – Provides in-house hematology and serum chemistries (contact the Pathology/Histotechnology Lab at 301-846-1281). Please see Appendices “Pathology/Histotechnology Laboratory Services” for additional information.

Necropsy - Necropsies on rodents are conducted at the AHDL for routine and clinical health evaluations. Necropsy procedures involve gross postmortem examination and the collection of tissues and other materials for serologic, bacteriologic, and parasitologic testing. The NCI-Frederick Pathology/ Histotechnology Laboratory

provides histopathologic support. Clinical cases and other cases that require immediate attention are given priority and are rapidly reported with verbal communication. Please also visit the Pathology/Histotechnology Laboratory website at <http://web.ncifcrf.gov/rtp/lasp/phl/>

## *NCI-Frederick Animal Holding and Technical Support Program*

### **GENERAL INFORMATION**

The Animal Holding and Technical Support Program provides the highest quality animal care and animal support services for all animal research at NCI-Frederick.

The functions of the Animal Holding and Technical Support Program are as follows:

- (1) Ensure that the investigator's needs are met by providing them with appropriately healthy research animals;
- (2) Ensure that all research animals are housed, handled, and cared for in a humane manner;
- (3) Provide a controlled research environment for all research animals; and
- (4) Provide scientific support for researchers performing animal-based research.

LASP serves as the central point to meet, address the needs, and support the animal requirements of both government and contractors in biomedical research activities of the NCI-Frederick. LASP works closely with NCI-Frederick Institutional Officials and the NCI-Frederick Institutional Animal Care and Use Committee (ACUC) in developing, implementing, and monitoring the programs and facilities of the NCI-Frederick for activities involving animals. The LASP also has the unique capability of conducting an investigator's animal facility research activities on their behalf.

Please note that all breeding and/or technical procedures must be included in the ACUC approved animal study proposal, not limited to, but including: injection details (volume/route/dose/frequency), time on study, number of animals, endpoints, surgical procedures, etc. No individual (animal user, caretaker, technician, investigator, etc.) is permitted to conduct procedures on animals unless the procedure has been previously reviewed and approved by the NCI-Frederick Animal Care and Use Committee. If during the course of your study, an investigator requires revisions or additions to his/her experimental research projects, he/she must submit a modification for review and approval by the NCI-Frederick ACUC before proceeding. Please contact the ACUC Coordinator if you have any questions regarding this issue.

### **CAGE ALLOCATIONS AND FACILITY RESOURCES**

All studies at the NCI-Frederick are scheduled through animal facility management to ensure that the necessary resources [technical staff, equipment, etc.] are available to support the proposed research project. In addition, all projects must be coordinated in advance to ensure that animal activities are maintained within an investigator's assigned cage allocation.

### **SECURITY**

Animal facilities present an atypical security risk due to the possibility of animal rights activists attempting to disrupt animal research facilities.

To counter this risk we have the following measures currently in place:

- (1) Protective Services Officers are on-duty 24-hours a day monitoring intrusion and equipment alarms;
- (2) Physical barriers requiring cardkey\* access and secured doors requiring keys\*\* or code access are in place in all animal facilities;

- (3) Locked areas and cabinets are used to secure potentially hazardous and sensitive materials (i.e., radioisotopes, chemical carcinogens, syringes, and needles); and
  - (4) Maintenance personnel are available, on-site, 24-hours a day to respond to any environmental alarms. The computerized alarm system continually monitors such factors as temperature, humidity, airflow, automatic animal watering, etc.
- \* Cardkeys can be obtained through a facility manager and are issued by Protective Services. ***If an investigator or a technician would like regular access to a facility area, they should apply for a cardkey to be issued as soon as possible.***
- \*\* Traditional metal keys are needed to get regular access to many facility areas. These can also be obtained through a facility manager and are also issued by Protective Services. ***As noted above, any investigator or technician that would like to have regular access to a facility area, they should apply for needed keys as soon as possible.***

## OVERVIEW OF LAWS & REGULATIONS

Research laboratories (animal facilities are included by definition) are not normally considered an area of pervasive federal regulation. However, there are many federal and state regulations that do apply to research laboratories. These regulations may be either of general or contractual application. General applications are those that must be obeyed by everyone. Violation of a general regulation may subject the violator to either civil or criminal penalties including fines and, under rare circumstances, incarceration. A contractual regulation is applied only by an agreement. Violation of the contractual regulations normally can only result in loss of the funding for the contract or grant.

## SPACE RECOMMENDATIONS FOR LABORATORY ANIMALS

Space recommendations for laboratory animals are identified in the *Guide for the Care and Use of Laboratory Animals*, 1996 revision, pp. 25-31, as reprinted below:

"An animal's space needs are complex, and consideration of only the animal's body weight or surface area is insufficient. Therefore, the space recommendations presented here are based on professional judgment and experience and should be considered as recommendations of appropriate cage sizes for animals under conditions commonly found in laboratory animal housing facilities. Vertical height, structuring of the space, and enrichments can clearly affect animals' use of space. Some species benefit more from wall space (e.g., "thigmotactic" rodents), shelters (e.g., some New World primates), or cage complexities (e.g., cats and chimpanzees) than from simple increases in floor space (Anzaldo and others 1994; Stricklin 1995). Thus, basing cage-size recommendations on floor space alone is inadequate. In this regard, the *Guide* might differ from the AWRs (see footnote 1, p.2).

Space allocations should be reviewed and modified as necessary to address individual housing situations and animal needs (for example, for prenatal and postnatal care, obese animals, and group or individual housing). Such animal-performance indexes as health, reproduction, growth, behavior, activity, and use of space can be used to assess the adequacy of housing. At a minimum, an animal must have enough space to turn around and to express normal postural adjustments, must have ready access to food and water, and must have enough clean-bedded or unobstructed area to move and rest in. For cats, a raised resting surface should be included in the cage. Raised resting surfaces or perches are also often desirable for dogs and non-human primates. Low resting surfaces that do not allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space. Floor space taken up by food bowls, water containers, litter boxes, or other devices not intended for movement or resting should not be considered part of the floor space.

The need for and type of adjustments in the amounts of primary enclosure space recommended in the tables that follow should be approved at the institutional level by the ACUC and should be based on the performance outcomes described in the preceding paragraph with due consideration of the AWRs and PHS Policy (see footnote 1, p. 2). Professional judgment, surveys of the literature and current practices, and consideration of the animals'

physical, behavioral, and social needs and of the nature of the proposal and its requirements might be necessary (see Crockett and others 1993, 1995). Assessment of animals' space needs should be a continuing process. With the passage of time or long-term proposals, adjustments in floor space and height should be considered and modified as necessary."

Footnote 1, p. 2 - Users are reminded that the Guide is written for a diverse group of national and international institutions and organizations, many of which are covered by neither the AWRs nor the PHS Policy. On a few matters, the Guide differs from the AWRs and the PHS Policy; users regulated by the AWRs or the PHS Policy must comply with them.

### *Minimum Space Recommendations for Laboratory Animals*

The chart below is also reprinted from the *Guide for the Care and Use of Laboratory Animals* and is a good reference on how your research animals need to be properly housed. Please remember that mice are the most commonly used research model at the NCI-Frederick and are generally housed at a maximum of five per cage for the "shoebox" style caging that is currently used in the NCI-Frederick animal facilities. All breeding colonies are maintained in accordance with LASP SOP 3.021 "Rodent Breeding and Weaning." Investigators are permitted to request exemptions to this SOP [please refer to the Guidance for Investigators Seeking Exemption to the Rodent Breeding and Weaning Policy at <http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines/ACUC40BreedingExempt.pdf>].

**Effective April 2003, all animals that are singly housed animals for greater than 90 days must be provided with an enrichment device.** The type of enrichment device will be determined by the facility management in conjunction with the investigative staff. Please keep in mind that for certain studies (e.g., behavioral) the ACUC understands that the use of environmental enrichment devices may provide unwanted variables in your research results. Any studies that do not wish to utilize enrichment devices must provide justification to the ACUC on the Animal Study Proposal Form (a new section will be added to the form). Exclusions for the use of enrichment devices will be made by the ACUC on a case-by-case basis.

ANIMAL	WEIGHT	HOUSING TYPE	FLOOR AREA/ANIMAL		HEIGHT	
			in <sup>2</sup>	cm <sup>2</sup>	in	cm
Mice	<10	Cage	6.0	38.71	5	12.70
	10-15	Cage	8.0	51.62	5	12.70
	15-25	Cage	12.0	77.42	5	12.70
	>25	Cage	15.0	96.78	5	12.70
Rats	<100	Cage	17.0	109.68	7	17.78
	100-200	Cage	23.0	148.40	7	17.78
	200-300	Cage	29.0	187.11	7	17.78
	300-400	Cage	40.0	258.08	7	17.78
	400-500	Cage	60.0	387.12	7	17.78
	>500	Cage	70.0	451.64	7	17.78

## *Safety and Environmental Compliance*

The Environment, Health, and Safety (EHS) Program is a technical resource for the NCI-Frederick, the function of which is to provide a safe working environment through the achievement of facility-wide compliance to applicable safety and environmental regulations. In research activities utilizing animals, factors supplemental to those of bench laboratory activities may need to be addressed to fulfill regulatory compliance. The initial mechanism employed to address such factors is by EHS's review of all Animal Study Proposals (ASP). In this process, EHS staff assesses each ASP bearing in mind the potential consequences of the proposed use of biohazardous agents, toxic chemicals, ionizing radiation, or recombinant DNA.

### **USE OF HAZARDOUS AGENTS AT NCI-FREDERICK ANIMAL FACILITIES**

#### Biohazardous Agents and Recombinant Materials

EHS maintains a registry of all research activities at the NCI-Frederick entailing the use of recombinant materials, human pathogens, human blood or other materials potentially infectious to humans. A key component of the registration process is the investigator's development of a proposal to specify relevant safeguards to deal with the proposed use of these items. The biosafety office within EHS functions as a resource in the development of the proposal and ultimately endorses the registration document. When an investigator proposes the use of rDNA or biohazardous agents in a study involving animals, a copy of the Institutional Biosafety Committee (IBC) Registration Document is to be included as part of the ASP. If at the time of ASP submittal, the investigator has not registered the material, then the investigator should contact the biosafety office to initiate the IBC registration process concurrent with the ASP. The ASP will not receive final EHS approval until the work with pathogens in animals has been approved by the IBC. Work with transgenic or knock-out animal models require review by the IBC.

#### Chemical Hazards

To meet OSHA regulations on hazard communication, NCI-Frederick employees must have ready access to information on the toxicity and other potential hazards of materials used in the performance of their duties. Therefore, in a proposal for the administration of toxic chemicals to animals, the investigator should list these materials on the ASP and append copies of available Material Safety Data Sheets (MSDS) or other relevant toxicologic information. When an ASP does not include MSDS, this information consequently is compiled by the industrial hygiene office during review, this may entail a holdup in the approval process. As part of the ASP review, EHS staff assesses whether the proposed animal facility incorporates engineering controls (hoods, directional airflow, waste collection provisions, etc.) necessary to protect animal facility staff from chemical exposure and to prevent the environmental release of toxic materials. When investigators propose work that entails the use of carcinogens, compliance to the NIH Guidelines for the Laboratory Use of Chemical Carcinogens and relative facility SOPs are required and is so indicated on the ASP during review by EHS staff.

#### Fire, Life Safety and Physical Hazards

Collaboratively, EHS staff, the U.S. Army Garrison Fire Department, and the facility's Occupant Evacuation Coordinator (formerly Fire Marshall), regularly inspect compliance of animal facilities to fire prevention codes, emergency egress provisions, and related life safety hazards. Normally, fire prevention code compliance inspections are conducted following a predetermined schedule rather than at time of ASP review.

#### Radiological Hazards

Historically, the use of ionizing radiation and radioisotopes with experimental animals at NCI-Frederick has been infrequent. Never-the-less, when the administration of radioisotopes is proposed in animals, the same surveillance, monitoring, and inspection programs required in laboratories are applied in the animal facilities. This entails these elements: the wearing of film badges by relevant individuals, the collection of urine bioassays on persons using P-32 or H-3, and thyroid scans for those persons using radioiodine. When an investigator proposes

the use of radioisotopes in a study involving animals, the radiation safety office ascertains that individuals working in that animal facility are appropriately trained and registered to use the specific isotope. Similarly, experiments involving irradiation of research animals are limited to those individuals trained and approved to utilize the Cs irradiators.

### Recombinant DNA

Following the NIH Guidelines for Research Involving rDNA Molecules (June 1994), all research activities that entail the use of rDNA are required to be registered with EHS. In a process analogous to the registration of biohazardous agents, the rDNA registration process comprises the development of a proposal addressing relevant safeguards. The biosafety office acts as a resource in the development of the proposal, but ultimate approval of nonexempt rDNA proposals is by the NCI-Frederick Institutional Biosafety Committee. When an investigator proposes the use of recombinant molecules in a study involving animals, a copy of the rDNA Registration Document is to be included as part of the ASP. If at the time of ASP submittal, the investigator has not registered the recombinant work, then the investigator should contact the biosafety office to initiate the rDNA registration process concurrent with the ASP. The ASP will not receive EHS approval until the work with pathogens in animals has been approved by the IBC. *Please review the guidance Animal Study Proposal Renewals and IBC requirements at [http://web.ncifcrf.gov/ntp/lasp/intra/acuc/fred/guidelines/IBC\\_ACUCDocuments.pdf](http://web.ncifcrf.gov/ntp/lasp/intra/acuc/fred/guidelines/IBC_ACUCDocuments.pdf)*

## **SAFEGUARDS IN NCI-FREDERICK ANIMAL FACILITIES**

### Safe Practices

The key element in maintaining a safe work environment for NCI-Frederick staff is that every employee uses safe techniques at all times. There is no better way to eliminate potential accidents than through personal effort and attention to the job at hand. Basic safety rules are presented in the NCI-Frederick Safety Manuals and in the LASP Standard Operating Procedures Manual. More specific safety practices can be presented in the proposals appended to ASPs or in MSDSs. Questions as to safe work practices should be immediately directed to one's supervisor or to EHS.

### Training

To increase the safety and health awareness of employees, a three-phase approach to safety training is employed at NCI-Frederick. All employees are provided initial safety orientation by EHS staff during their first month on the job. Also as part of the new employee's check-in process, the supervisor instructs the new employee of all the potential hazards, safety procedures, emergency procedures, and safeguards associated with their tasks. Such supervisory training is a continuing process where new information on hazards is provided to the employee as new potential hazards are introduced. In addition, EHS and OHS provide safety and health training on a variety of issues throughout the year.

### Safety Equipment

Personnel entering an animal facility are required to wear safety gear and special clothing to provide protection to the worker's health, the animal's health, or both. Such protective clothing may be either long-sleeved scrubs or long-sleeved coveralls. Disposable gloves, shoe covers, head covers, and facemasks are provided to be worn when entering animal holding areas and when handling animals, according to facility operating procedure. Nitrile gloves are preferred over latex gloves due to the risk of latex-induced allergic reactions.

Steel-toed safety shoes/boots are furnished and required to be worn by all employees working in foot hazard areas of animal facilities. Disposable shoe covers may be required according to facility operating procedure.

Prescription or nonprescription safety eyewear is provided to all employees working in eye hazard areas of the animal facility. Safety eyewear must meet the requirements of the most recent issuance of ANSI Z-87 and must incorporate side shield protection. In some work situations, such as the handling of concentrated corrosive liquids, full-face protection may be necessary. Emergency showers and eyewash stations are provided for drenching skin or eyes in the event of chemical contamination. FME staff flush emergency showers quarterly.

Eyewash stations must be flushed monthly (and preferably weekly) by the user to purge the plumbing lines and to verify proper operation.

The wearing of respiratory protective devices is an infrequent requirement limited to a few select areas of animal facilities where ventilation may not reduce airborne hazards to an acceptable level. Such would be indicated on the ASP proposal or by the facility operating procedure. It is important to note that surgical masks worn in animal facilities provide no protection to the wearer; rather, they are worn for the protection of animal health. Since there is no universal respirator, it is critical that one complies with EHS's specification of the appropriate type of respirator and the frequency for replacement of expendable components. Personnel must be medically certified by OHS prior to wearing of respiratory protective equipment. Annual fit-testing and training is also mandatory for all employees approved to wear respirators.

Some cage washing areas in animal facilities may expose workers to sound intensity levels greater than the 8-hour time-weighted action level of 85 dB. This noise evaluation is made by EHS staff in conformance with OSHA regulation. If the evaluation indicates the area to comprise a noise exposure hazard, then persons working in the noise hazard area will be provided and must wear hearing protection devices (ear muffs or ear plugs).

### Facility/Engineering Controls

The animal facilities at NCI-Frederick incorporate varying degrees of engineering safeguards designed to provide protection to animal and human occupants. At the time of ASP safety review, particular attention is given to ensure that the proposed facility comprises the sufficient degree of facility/engineering controls necessary to safely conduct investigations with the hazardous agents. In some situations, relocating the proposed animal work to a facility that provides a higher degree of protection may be necessary.

In terms of biosafety considerations, research animal facilities can be regarded as extensions of research laboratories, thus CDC/NIH Biosafety in Microbiological and Biomedical Laboratories specifies four combinations of practices, equipment, and facilities for experiments on animals that may produce human infections. These combinations provide increasing levels of protection and are designated animal biosafety levels (ABSL) -- animal facilities at NCI-Frederick do not surpass ABSL 2. Pertinent areas of animal facilities are formally inspected annually by EHS staff to renew ABSL registration to include proper functioning of biosafety cabinets, autoclaves, exhaust ventilation, etc. as appropriate to the ABSL.

In terms of chemical safety, the NIH Guidelines for Laboratory Use of Animal Carcinogens specifies facility standards necessary when using potentially carcinogenic compounds. These facility standards are equally valid for work with highly toxic chemicals that are not carcinogenic. Similarly, the radiation safety manual specifies facility criteria for the administration of radioisotopes to animals.

### Safety Inspections and Regulatory Audits

All facilities at the NCI-Frederick, including animal facilities, are formally inspected at least annually by EHS staff. For most animal facilities these inspections are coincident with the semiannual ACUC inspections. Animal areas registered to use pathogenic agents or to perform recombinant work are provided supplementary semiannual assessments by the biosafety office. Animal facilities that generate either chemical or radiological wastes are subject to frequent informal inspections as a part of the waste pickup service by EHS staff. The US Nuclear Regulatory Commission visits the NCI-Frederick to conduct unannounced radiation program audits. All research and support facilities are susceptible to unannounced compliance audits by agents of EPA and OSHA. Those areas using controlled substances are subject to unannounced inspection by licensed agents of the US Drug Enforcement Administration.



## Monitoring

To evaluate potential occupational exposure of NCI-Frederick employees to hazardous agents, EHS staff conducts a number of monitoring activities. Those areas that are part of radiation programs are provided with monthly surface contamination surveys by EHS staff collecting and analyzing wipes taken from appropriate areas within the animal and/or treatment room. Radiologic drain contamination surveys are also conducted. Similar monitoring for chemical exposures is conducted by EHS on an as-needed or as-requested basis. In addition, EHS staff conducts employee noise dosimetry and annual sound intensity measurements in facilities with large tunnel cage washers to quantify the existence of noise hazard areas and the necessity for hearing protection devices.

Any NCI-Frederick employee concerned with potential occupational exposures is encouraged to contact EHS to arrange for a work practice evaluation and subsequent exposure monitoring, as warranted by EHS.

## *Occupational Health Program*

Occupational Health Services (OHS) conducts a comprehensive occupational health program for the NCI-Frederick. With emphasis on prevention, the purpose is to:

- Improve employee wellness;
- Increase job satisfaction and productivity;
- Comply with Federal and State regulations; and
- Decrease costs associated with absenteeism, disability, worker's compensation and health insurance

The occupational health program is essential for employees working in laboratories, facilities maintenance, animal facilities, and for employees with animal contact. Disease/accident prevention, treatment, management and rehabilitation are components of the program to limit the potential for disability or adverse sequelae among the employees working at the facility. Health promotion, multi-faceted screening and surveillance programs, workers' compensation case management and employee assistance programs are provided. OHS works closely with the Occupational Safety Branch of EHS and other expert resources to ensure that employees receive the benefit of various disciplines and perspectives in managing occupational health care.

The surveillance program for employees includes an initial comprehensive medical evaluation (post-offer, pre-employment) to obtain baseline information and to ensure proper job placement. Periodic medical evaluations are provided for health assessment, surveillance and screening, and to encourage maintenance of good health.

The medical evaluation includes:

- Medical and work history
- Tuberculin skin testing
- Visual examination
- Audiometric examination
- Chest x-ray, ECG, as indicated
- Initial serum (for banking) Physical examination
- Respirator fit testing and surveillance

Animal allergen exposure is an important part of the initial and periodical medical health evaluations. Allergen exposure is addressed multiple times in the new hire and transfer health history evaluation under the following sections: past medical history, review of symptoms, social history, and occupational history. The interval health history is also offered annually and contains direct questions pertaining to changes in health history and allergies.

Occupational Health Services is staffed by nurse practitioners, registered nurses, an employee assistance professional, and highly trained support staff. A consulting physician is in regular attendance and on call at other times. The group is located in Building 426 on the NCI-Frederick campus and occupies several offices and

examination rooms. The nurse practitioners and nurses are available for emergencies during regular working hours in OHS and on site, after hours by pager through Protective Services. The staff are available for consultations from 8:15 AM – 5:00 PM on and off site through the front desk, at 301-846-1096.

## *References*

Alternatives to Animal Use in Research, Testing, and Education. Congress of the United States, Office of Technology Assessment. (Washington, DC: 1986 / OTA-BA-273).

Anatomy of the Laboratory Rat. R. Hebel and M.W. Stromberg. (Baltimore: The Williams and Wilkins Co., 1976).

Anesthesia and Analgesia in Laboratory Animals. D. Kohn, S. Wixson, W. White, and G.J. Benson, eds.; American College of Laboratory Animal Medicine Series. (New York: Academic Press, 1997).

Animal Physiologic Surgery, 2nd ed. C.M. Lang, ed. (New York: Springer-Verlag, 1982).

The Biology and Medicine of Rabbits and Rodents, 2nd ed. J.E. Harkness and J.E. Wagner. (Philadelphia: Lea and Febiger, 1983).

The Biomedical Investigators Handbook for Researchers Using Animal Models. Foundation for Biomedical Research. (Washington, DC, 1987).

Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals. B.M. Mitruka and H.M. Rawsley. (New York: Masson Publishing USA, Inc., 1977).

Clinical Laboratory Animal Medicine - An Introduction. Donald D. Holmes. (Ames: Iowa State University Press, 1984).

Control of Communicable Diseases in Man, 16th ed. Abram S. Benenson, ed. (Washington, DC: American Public Health Association, 1995).

Current Veterinary Therapy IX - Small Animal Practice. R.W. Kirk, ed. (Philadelphia: W.B. Saunders, 1986).

Current Veterinary Therapy - Food Animal Practice. J.L. Howard, ed. (Philadelphia: W.B. Saunders, 1981).

Experimental Surgery: Including Surgical Physiology, 5th ed. J. Markowitz, J. Archibald, and H.G. Downie. (Baltimore: Williams and Wilkins, 1964).

Formulary for Laboratory Animals, 2<sup>nd</sup> ed. T. Hawk and S. Leary. (Ames: Iowa State University Press, 1999).

The Future of Animals, Cells, Models and Systems in Research, Development, Education, and Testing. National Academy of Sciences. (Washington, DC: 1977).

Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (Revised 1996).

Guide to the Care and Use of Experimental Animals, 2 volumes. Canadian Council on Animal Care. (Ottawa, Ontario: 1993).

Handbook of Laboratory Animal Science, 3 volumes. E.C. Melby, Jr. and N.H. Altman. (Cleveland: CRC Press, 1974 and 1976).

Laboratory Animal Anesthesia: An Introduction for Research Workers and Technicians. P.A. Flecknell. (San Diego: Academic Press, 1987).

Laboratory Animal Medicine. J.G. Fox, B.J. Cohen, and F.M. Loew, eds.; American College of Laboratory Animal Medicine Series. (Orlando: Academic Press, 1984).

The Laboratory Rat. H.J. Baker, J.R. Lindsey, and S.H. Weisbroth, eds.; American College of Laboratory Animal Medicine Series; Vol. I - Biology and Diseases (1979) and Vol. II - Research Applications (1980). (Orlando: Academic Press).

Man and Mouse - Animals in Medical Research. William D.M. Paton. (Oxford and New York: Oxford University Press, 1984).

The Merck Veterinary Manual, 6th ed. C.M. Fraser, ed. (Rahway, NJ: Merck and Co., Inc., 1986).

Models of Human Disease: Handbook, The Registry of Comparative Pathology. Armed Forces Institute of Pathology. (Washington, DC: 1985).

The Mouse in Biomedical Research. H.L. Foster, J.D. Small, and J.G. Fox, eds.; American College of Laboratory Animal Medicine Series; Vol. I - History, Genetics, and Wild Mice (1981), Vol. II - Diseases (1982), Vol. III - Normative Biology, Immunology, and Husbandry (1983), and Vol. IV - Experimental Biology and Oncology (1982). (Orlando: Academic Press).

Nutrient Requirements of Laboratory Animals, 3rd rev. ed.; Nutrient Requirements of Domestic Animals Series (no. 10). Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture and Renewable Resources, National Research Council. (Washington, DC: National Academy of Sciences, 1978).

Outline of Veterinary Clinical Pathology, 3rd ed. M.M. Benjamin. (Ames: Iowa State University Press, 1978).

Parasites of Laboratory Animals, 2 volumes. R.J. Flynn. (Ames: Iowa State University Press, 1973).

Pathology of Aging Rats - A Morphological and Experimental Study of the Age Associated Lesions in Aging BN/Bi, WAG/Rij, and (WAG x BF)F1 Rats. J.D. Burek. (West Palm Beach, FL: CRC Press, 1978).

Pathology of Laboratory Animals, 2 volumes. K. Benirschke, F.M. Garner, and T.C. Jones. (New York: Springer Verlag, 1978).

The Pathology of Laboratory Animals. W.E. Ribelin and J.R. McCoy, eds. (Springfield, IL: Charles C. Thomas, 1965).

The Principals of Humane Experimental Technique. W.M.S. Russell, and R.L. Burch. (London: Methuen and Co. Ltd., 1959).

Principles of Proper Laboratory Animal Use in Research. G.R. Novak, ed. (Silver Spring, MD: MTM Associates, Inc., 1987).

Public Health Service Policy on Humane Care and Use of Laboratory Animals. Office for the Protection from Research Risks, National Institutes of Health. (Rockville, MD: revised 1986).

AVMA Guidelines on Euthanasia [2007]. [http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf)

Textbook of Veterinary Anesthesia. L.R. Soma, ed. (Baltimore: Williams and Wilkins, 1971).

Trends in Bioassay Methodology: In Vivo, In Vitro and Mathematical Approaches. NIH Publication No. 82-2382, (1982).

The UFAW Handbook on the Care and Management of Laboratory Animals, 6th ed. T.B. Poole ed. (Essex: Longman Group UK Limited, 1987).

Veterinary Anesthesia, 2nd ed. W.V. Lumb and E.W. Jones. (Philadelphia: Lea and Febiger, 1984).

Veterinary Pathology, 5th ed. T.C. Jones and R.D. Hunt. (Philadelphia: Lea and Febiger, 1983).

Veterinary Pharmaceuticals and Biologicals. C.E. Aronson, ed. (Edwardsville, KS: Veterinary Medicine Publishing Co., 1984).

Veterinary Pharmacology and Therapeutics, 5th ed. L.M. Jones, N.H. Booth, and L.E. McDonald. (Ames: Iowa State University Press, 1982).