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I. Introduction  
   A. Purpose  

This manual describes the resources available for animal research at the University of Southern California. It is intended to provide information to principal investigators, research staff, and students regarding: 1) the organization and administrative lines of authority regarding animal care and use at USC, 2) the laws, regulations, policies, and other accepted standards pertaining to appropriate
animal care and use in research and teaching, 3) the mechanism to obtain approval for animal use through the Institutional Animal Care and Use Committee, and 4) the policies, procedures, and services of the Department of Animal Resources by which animal care standards are administered to meet the needs of the USC research community.

B. Investigator Responsibilities

The use of animals for research and teaching is a fundamental necessity for continued progress in the biological and medical sciences. Such use of animals is a privilege to the scientific community. Therefore, each investigator and member of his or her staff is responsible to fulfill all ethical and legal requirements. USC faculty members who use animals in their research or teaching activities are accountable to federal, state, and local laws, regulations, and policies governing animal use. All activities involving the use of animals are subject to oversight by the Institutional Animal Care and Use Committee (IACUC).

These regulations and policies cover the following:

- Preparation and submission of protocols for the use of animals
- Acquisition, care and justification of the use of appropriate species and numbers of animals
- Training of personnel in appropriate methods of animal experimentation
- Minimization or avoidance of animal pain and distress in concert with sound scientific practices
- Consideration of alternatives to animal use
- Pre-surgical evaluation, surgical methods, and post-operative care
- Disclosure of experimental endpoints
- Occupational health and safety for persons working with animals
- Euthanasia of animals

C. Limitations of Liability

To the maximum extent permitted by law, in no event will the USC Department of Animal Resources, the Institutional Animal Care and Use Committee and the Office of the vice President for Research be responsible for any incidental damages, consequential damages, exemplary damages of any kind, lost goodwill, lost research, and/or any indirect economic damages whatsoever regardless of whether such damages arise from claims based upon contract, negligence, tort (including strict liability or other legal theory), a breach of any warranty, or any other actions, and regardless of whether a party was advised or had reason to know of the possibility of incurring such damages in advance.
II. Organization

A. Administrative Line of Authority

The Institutional Official (IO) has overall responsibility for all aspects of compliance with animal welfare regulations and policies. The Vice President for Research is the Institutional Official at USC. The Institutional Animal Care and Use Committee and the Director of the Department of Animal Resources report directly to the IO.

B. Institutional Animal Care and Use Committee (IACUC)

The Health Research Extension Act and the Animal Welfare Act (see section below regarding laws) require the IO to appoint an Institutional Animal Care and Use Committee to oversee the institution’s animal care and use program. The IACUC membership must include an individual unaffiliated with the institution, a veterinarian who has direct authority and program responsibilities with the University, a practicing scientist experienced in research involving animals, and a member who is not a scientist. The USC IACUC consists of individuals who collectively meet these requirements.

The functions of the IACUC, which are defined by regulations, are as follows:

At least twice per year, the USC IACUC must:

1. Review the Institution’s program for the humane care and use of animals.

2. Perform inspections of all institutional animal facilities and study areas.

3. Prepare reports of the inspections, including departures and deficiencies from accepted regulations and their identification as major or minor deficiencies.

In addition, the USC IACUC must:

4. Review all proposals (protocols) for the use of vertebrate animals in research and education.

5. Review specific complaints or concerns about animal care and use.

6. Impose suspensions and sanctions for investigators who do not comply with policies.

7. Make recommendations to the IO regarding any aspect of the Institution’s animal program, facilities, and personnel training.
C. Department of Animal Resources

Animal Resources is the university-wide department that provides animal procurement, daily care, animal health care, and scientific support for USC programs using animals in teaching and research.

Adequate veterinary care must be provided to all animals as required by regulations. This includes access to all animals for evaluation of their health and well being. In addition, the institutional attending veterinarian has the authority to oversee the adequacy of other aspects of animal care and use, including animal husbandry, nutrition, sanitation practices, zoonosis control and hazard containment. The attending veterinarian for the university is the Director of the Department of Animal Resources.
III. Regulations, Policies, and Guidelines

This section contains information on regulations, policies, guidelines, and other standards relevant to animal research at USC. General policies such as U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training are reprinted in total in this section. Other regulations and guidelines are paraphrased. Complete copies of these documents are available from the office of the Director of Animal Resources.

A. 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 the 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 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 care and use committee. Such exceptions should not be made solely for the purpose of teaching or demonstration.

B. The Health Research Extension Act and Public Health Service Policy

The Health Research Extension Act (PL 99-158, 1985) incorporates the U.S. Government Principles and serves as the legislative mandate for animal welfare assurance at institutions that receive Public Health Service (PHS) funding. The Public Health Service Policy on Humane Care and Use of Laboratory Animals, requires each institution receiving PHS funds for research involving animals to file an Animal Welfare Assurance Statement with the PHS, Office of Laboratory Animal Welfare (OLAW). This statement commits the institution to compliance with the: Animal Welfare Act, The Guide for The Care and Use of Laboratory Animals (The Guide) and the U.S. Government Principles for The Utilization and Care of Vertebrate Animals Used in Testing Research and Training. The statement must describe in detail the institutional program for the care and use of all vertebrate animals used in research, teaching or testing.

The PHS policy requires the Institutional Animal Care and Use Committee (IACUC) to approve the care and use of animals as proposed in PHS grant applications before funds will be awarded. The IACUC is also required to conduct semi-annual assessments of the institutional program for care and use of animals, using The Guide as a basis for evaluation. Significant deficiencies in the institution’s program must be identified, and the institution must adhere to an approved plan and schedule for correction of the deficiencies.

Failure by the institution to comply with these policies may lead to various actions, including the termination of PHS support for projects at the institution.
Approval of a protocol for the use of animals is communicated to the funding agency by the chair of the IACUC in an assurance letter, accompanied by the institutional animal welfare assurance identification number.

C. The Animal Welfare Act


The Animal Welfare Act is enforced by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). Research institutions and facilities are subject to unannounced inspections by the USDA veterinarians and are required to file an annual report listing the species and numbers used in research and certify that anesthetic, analgesics and tranquilizing drugs are used appropriately during research and testing. The AWA also specifies standards of animal care and transportation and requires the establishment of an IACUC.

Failure to comply with USDA standards may result in civil or criminal prosecution and suspension of animal research activities at the institution.

D. The Centers for Disease Control and Prevention (CDC)

The CDC publishes the guideline Biosafety in Microbiological and Biomedical Laboratories which provides requirements for work with biological agents which may be infectious to humans. The publication includes requirements for facilities, equipment, and work practices when working with animals infected with biological agents.

The CDC also regulates the importation of all non-human primates into the United States. Only organizations or individuals registered with the CDC may import non-human primates. U.S. importers must comply with CDC record keeping and reporting requirements.

E. The Fish and Wildlife Service (Department of the Interior)

The Fish and Wildlife Service is a federal agency in charge of enforcing the Endangered Species Act and The Convention of International Trade in Endangered Species of Fauna and Flora (CITES). Institutions which seek to use species covered by the Act for scientific research must obtain permits from The Federal Wildlife Permit office. If an institution intends to import or export an endangered or threatened species; appropriate documents must be obtained in advance as required by CITES.
F. The Animal and Plant Inspection Service (APHIS)

In addition to enforcing the Animal Welfare Act regulations, this office of the United States Department of Agriculture (USDA) requires that individuals who intend to import materials of animal origin into the United States must obtain an appropriate permit. Permits may be required for a range of biological materials including cell lines and culture media containing animal serum.

G. The Drug Enforcement Act (P.L. 93-205)

The Drug Enforcement Act is enforced by the Drug Enforcement Administration (DEA) of the Department of Justice. This Act requires appropriate security and record management of substances considered to be potentially addictive or habituating.

H. The Food and Drug Administration (FDA)

The Food and Drug Administration sets standards for testing of foods, drugs and other chemicals, which will be used by or come into contact with humans. Federal regulations require animal testing for safety and efficacy before the substances are approved for clinical trials in humans. The FDA has established Good Laboratory Practices (GLP) regulations for this purpose. The regulations require extensive documentation and quality assurance procedures for animal care, including quarantine and isolation, disease diagnosis, animal identification, routine animal husbandry, caging, sanitation, training and qualification of personnel involved with animals.

I. State Agencies and Regulations

The California Department of Fish and Game administers laws regulating the importation, quarantine and housing of non-dangerous wildlife species and the collection and use of native California species (California Administration Code, Title 14, Sections 671-671.4). Institutions seeking to import restricted or prohibited species for research must obtain the appropriate permits through the Animal Welfare section of this department. These regulations are intended to protect native California wildlife and the public from diseases, which may be carried by imported wildlife. Certain laboratory species such as Xenopus frogs, ferrets and gerbils are covered by these regulations. Also, any transgenic aquatic animals must be maintained under strict conditions to prevent release into the wild. Permits must be obtained to maintain these animals for research.

The California Department of Health Services is charged with investigating Reportable infections, or human diseases that have a possible zoonotic source. This agency is also responsible for the regulation of importation of non-native birds and mammals into California. The department publishes a list of species which can only be imported by permit and sets quarantine requirements for non-native and non-domestic species. In particular, carnivores which are neither native nor domestic must be quarantined for a period of 90 days, and non-human
primates must be quarantined for at least 30 days with completion of at least two tuberculin tests.

The California Occupational Safety and Health Administration (CAL OSHA) administers regulations in detail pertaining to employee occupational health and safety including potential health hazards to persons who work with animals.

J. Local Agency

The City of Los Angeles Animal Regulation issues permits for facilities housing dogs, cats and wild species including nonhuman primates. City officials also inspect USC animal facilities once per year.

K. Accreditation of USC Facilities

Since 1966, USC has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). AAALAC is a non-profit organization established by scientific and educational organizations to ensure high standards of laboratory animal care and use. Accredited facilities must submit an annual report and have their program and facilities evaluated at least every three years. The AAALAC accreditation process involves an assessment of all aspects of the animal care and use program, including compliance with all applicable law, regulations, and guidelines. The Guide for the Care and Use of Laboratory Animals is the primary document used for AAALAC evaluations.
IV. Grant and Protocol Preparation

A. Animal Welfare Statement

Some granting agencies request an animal welfare assurance statement within the grant, or a statement may be added at the option of the principal investigator. Following is a sample animal welfare assurance statement for grants and contracts:

"The University of Southern California (USC) is a leader in the ethical and humane use of animals for research and teaching. USC is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and has an animal welfare assurance (number A3518-01) on file with the NIH Office of Laboratory Animal Welfare. The Institutional Animal Care and Use Committee review all applications to ensure ethical and humane treatment of animals. This body follows the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and all applicable government regulations including those of the U.S. Department of Agriculture and the State of California."

B. Activities that Require IACUC Approval

Any USC faculty member who chooses to use vertebrate animals including traditional laboratory animals, farm animals, wildlife or aquatic animals must submit a Protocol Synopsis Form to the IACUC for review and approval. The principal investigator on the protocol synopsis must be a USC faculty member. Other non-faculty members (e.g., postdoctoral fellows) who wish to initiate research projects using animals must submit forms as a co-investigator, with a faculty member listed as the principal investigator. Forms are available at the Animal Resources Office and on the Department of Animal Resources and IACUC website. The IACUC is required to review and renew each protocol annually. In addition, the PHS requires a complete review of previously approved protocols every 3 years. This is accomplished by the submission, review, and approval of a complete Protocol Synopsis Form.

C. Instructions for completing the Protocol Synopsis Form

1. Animals Covered:

Covered animals include all live vertebrate animals used for research or teaching by faculty and students of the University of Southern California.

In some cases, the animal work may be part of a collaborative study with another institution. For example, the funding for the research may be granted to USC, but all or part of the animal work may be performed at another
institution. In other situations, animals may be transported to or from USC as part of a collaborative study. In these cases, the animal use must be approved by the USC IACUC and documentation of the approval from the IACUC at the collaborating institution must also be provided. In addition to protocol approval information, the health status of animals transported to and from other institutions must be documented (see the section on animal transfers and movements in Section IX of this manual).

2. Forms to be completed:

The Protocol Synopsis Form needs to be submitted by a principal investigator who holds a faculty appointment or joint faculty appointment at USC.

If the research is using animal tissues only which will be supplied by another investigator, a one-page Tissue Request Form is all that is required. The investigator responsible for ordering the animals must have a protocol approved by the IACUC and sign the Tissue Request Form agreeing to supply the specified tissue(s).

In some cases, funding agencies require IACUC approval for program grants, stipends, or other funding mechanisms that may have animal components, but the animal component may involve several investigators at USC. If no animals are to be ordered on the grant directly, the one-page Review Form for Stipends/Fellowships/and Program Project/Core Grants is required. An approval number, which is not valid for ordering or maintaining animals will be issued. Any investigators intending to use animals under the grant must then submit their own protocol synopsis forms if they are not already approved.

3. Review Process:

Protocol Synopsis Forms must be submitted to the IACUC on or before published deadlines each month in order to be reviewed during that month. For most months, this deadline is noon on the first working day of the month. Full committee approval and notification of external agencies takes place at monthly committee meetings.

4. Continuing Reviews:

Even though an investigator may be approved for a multiple-year project, the IACUC must review each protocol on an annual basis as required by the Animal Welfare Act regulations. Yearly updated information and any changes in the animal use protocol should be supplied on the Continuing Review Form.

Approvals will be extended for another year if there are no major changes in the protocol. In addition, the Public Health Service policy requires a complete review of activities every three years. To do this, a complete Protocol Synopsis Form must be completed and approved by the IACUC.
5. Amendments to Protocols:

If there are any changes to the protocol during the course of the research program or upon annual renewal, an amendment must be submitted to the IACUC for approval. The **Continuing Review Form** is used for minor amendments. Minor amendments can be approved by a sub-committee of the IACUC, appointed by the IACUC chairman. A list of approved amended protocols will be presented to the full committee meeting. The following minor changes to an approved IACUC protocol can be submitted to the IACUC on a Continuing Review Form with an attached memorandum describing the change:

a) change of title
b) change of funding agency (a copy of the grant must be included)
c) addition or deletion of personnel
d) change of the animal strain (note a change in animal *species* must be submitted on complete form)
e) change of facility where the research will be conducted

These following changes are considered full amendments and require the submission of a **complete Protocol Synopsis Form**. Revised or added wording must be manually underlined or highlighted to be accepted by the IACUC:

a) change of species (note a change in *strain* within the same species is a minor change).
b) any change of procedures (e.g., methods of anesthesia, blood collection, experimental treatment, or euthanasia methods).
c) any addition of procedures (i.e. survival surgical manipulation or other procedures requiring anesthesia with recovery, procedures involving hazardous or infectious agents (provided the PI also shows evidence of approval from the appropriate safety committee).
d) increase in animal numbers with justification
e) transfer of the protocol to a new principal investigator (accompanied by written documentation from the previous PI that the research is being transferred to another PI).
The IACUC reserves the right to request a full committee review if deemed necessary for any protocol or protocol amendment.

6. Release of Funds and Procurement of Animals:

Administrators and staff have been instructed not to release funds or process animal orders unless an approval number or verification is present.

7. Release of Protocol Information:

Copies of protocol synopsis forms or any information on forms will be released only to the principal investigator or co-investigator(s) listed on the protocol synopsis form. The investigator or co-investigator may come to the Animal Resources office personally to pick up a copy. Protocols or related information may be sent by campus mail or picked up by a designated individual if a written, signed request from the investigator of co-investigator authorizes the release of information.

Protocols may be viewed with valid reason in the Animal Resources office by IACUC members, Animal Resources staff, and authorized regulatory or accreditation site visitors.

D. Instructions on Specific Sections of the Protocol Synopsis Form

1. Animal Use Classification:

Each protocol synopsis form must list classifications of animal use. This information assists the IACUC in evaluation of the animal use and becomes part of annual reporting to the United States Department of Agriculture. The IACUC may request that certain procedures be classified differently than originally listed on the form as appropriate. Following is a list of categories providing possible examples of procedures which are representative of each category.

Type A: Studies, which cause little or no pain or distress. Examples include standard approved methods of euthanasia that induce rapid unconsciousness (without surgical interventions prior to death of the animal). Also included are housing and brief restraint of animals for observation or examination; single blood sampling; single injections of non-toxic materials; short periods (a few hours) of food and water deprivation; and behavioral observations.

Type B: Studies, which may involve minor pain or distress of short duration, but where pain relieving drugs, are given as part of the study. Examples include surgical procedures and other studies on anesthetized animals where the animals do not regain consciousness (non-survival surgery) and surgical procedures where animals do regain consciousness (survival...
surgery) but where post-surgical pain and distress are expected to be minimal and analgesics are given to control pain as appropriate. Following all survival surgical procedures, it is expected that investigators adhere to acceptable veterinary practices including postoperative analgesia, fluid therapy and veterinary nursing care as appropriate. Also included in Category B are overnight or longer food or water deprivation; behavioral studies on awake animals that involve short-term restraint; studies using noxious stimuli from which escape is possible; using tumor implants or hybridomas under guidelines as outlined by the IACUC; and the use of Freund’s complete adjuvant under guidelines as outlined by the IACUC.

Comment: During and after Type B studies animals are not expected to show anorexia, dehydration, abnormal discharges, hyperactivity, increased recumbency or dormancy, increased vocalization, self-mutilation, aggressive-defensive behavior or demonstrate social withdrawal and self-isolation.

Type C: Studies, which may involve moderate pain or distress. These include major recovery surgical procedures performed under anesthesia where there is possible distress in animals even though analgesics are given to eliminate pain. Following all survival surgical procedures, it is expected that investigators adhere to acceptable veterinary practices including postoperative analgesia, fluid therapy and veterinary nursing care. Also included in Category C are studies involving prolonged periods (several hours or more) of physical restraint; prolonged deprivation of the animals’ environmental necessities, such as food or water; procedures which alter perceptual or motor functions, such as the induction of paralysis or seizures; and induction of infectious diseases or toxicities, and when severe clinical symptoms begin to appear the animals are treated or euthanized.

Comment: The IACUC normally requires monitoring of type C studies by veterinary staff and/or IACUC members. Involvement of trained technicians, scientists and veterinarians is critical if this pain is to be minimized or avoided. Animals used in Type C studies should not show signs of prolonged clinical distress, such as behavioral abnormalities, lack of grooming, dehydration, anemia, abnormal vocalization, prolonged anorexia, self-mutilation, increased signs of infectious processes (peritonitis, pneumonia, diarrhea, encephalitis, etc.). If these clinical abnormalities develop, the necessary treatments to alleviate the symptoms must be available and provided. If the symptoms cannot be alleviated, the animals must be euthanized with minimal delay.

Type D: Projects that may involve moderate to severe pain or distress without the benefit of pain-relieving drugs or other appropriate therapy. Such studies include application of noxious stimuli from which escape is impossible; exposure to noxious stimuli or agents whose effects are unknown; completely new experiments which have a high degree of invasiveness; induction of aggressive behavior leading to self-mutilation or fighting; and induction of infectious diseases or toxicities where death is an end point and
animals are not treated or euthanized when severe clinical abnormalities develop.

Comment: Type D projects present an explicit responsibility on the faculty to explore alternative methods before proceeding with the study. Type D projects are considered by some to be highly questionable or unacceptable, irrespective of the significance of the anticipated results. Before the IACUC can review and approve these projects, the justification statements and the veterinary involvement must be clearly presented.

2. Research personnel:

All personnel (faculty, staff, and students) who will use animals on the protocol must be listed with their 10-digit USC employee number. The USC security department requires one of these numbers for identification of staff who will work in animal facilities after hours and on weekends or holidays.

The training status of all staff must also be listed. Animal Resources training consists of information on animal research laws and guidelines and methods for proper animal care, handling and experimental manipulation. **Completion of training will be listed as a required stipulation of IACUC approval if it has not already been completed for the personnel listed.**

3. Adjuvants:

When an adjuvant is necessary, the use of Ribi Adjuvant, Incomplete Freund’s Adjuvant (IFA) or other adjuvants that cause less inflammation than Complete Freund’s Adjuvant (CFA) are desirable. The use of CFA may cause undesirable and painful side effects such as large inflammatory lesions or tissue necrosis. These can be effectively reduced or eliminated if the CFA is given only once, with booster immunizations given in another adjuvant or with no adjuvant. In addition, it is important to use appropriate routes of administration, adequate separation of injection sites, and the use of small amounts of inoculum per site. If it is absolutely necessary, CFA may be used under the conditions outlined below:

a) Injection of foot pads with CFA or other agents causing significant inflammation are only acceptable if scientifically proven to be necessary and if given a variance by the IACUC.

b) Inoculation of CFA intravenously or into lymph nodes is not acceptable.

c) CFA can only be used for the initial immunization unless given specific approval by the IACUC.
d) In rabbits or rodents subcutaneous injections will be performed at up to six sites on either side of the dorsal midline between the scapulae and the pelvis. In rabbits, a 2cm area of hair should be clipped and the skin prepared using povidone iodine followed by 70% ethanol. The number of sites should be minimized based on the total volume to be inoculated.

e) The site of injection must be monitored regularly (twice a week for 3 weeks) after injection for evidence of severe inflammation, large granuloma or abscess formation, and ulceration. The animal must be monitored regularly for evidence of pain, distress, or infection resulting from the injection.

f) The inoculation should be free of extraneous microbial contamination. Millipore filtration of the antigen before mixing with adjuvant is recommended when possible.

g) The injection site should be clean to prevent infection.

h) Incomplete Freund’s adjuvant, Ribi adjuvants, and other adjuvants may be employed using other reasonable injection protocols, including injections into lymph nodes or sites immediately draining into lymph nodes when antigen is very limited in quantity.

4. Tumor growth:

This applies to all studies that involve the induction of tumor growth in animals. This includes, but is not limited to, spontaneous, transplantable, chemically induced tumors and neoplasias in genetically manipulated rodents that may have increased incidence of a certain tumor type.

On the IACUC protocol synopsis form, the investigator should define a set of conditions under which the affected animals will be euthanized. The use of survival time as an endpoint should be avoided and will require a specific detailed justification for IACUC approval. Animals should be euthanized before their tumor burden becomes excessive and before the animals become debilitated.

Rodents in which tumors have been induced should be checked at least once daily. Assessment of pain, distress and discomfort, should be based on evaluating these five aspects of an animals condition:

- changes in body weight (and related changes in food and water intake)
- external physical appearance (e.g. ulcerated, enlarged tumors that interfere with normal movement)
• observable clinical signs (e.g. change in breathing, emaciation, abnormal discharges, diarrhea, hunched posture)

• changes in behavior (e.g. excessive sleeping, self inflicted trauma)

• changes in behavioral responses to external stimuli (e.g. either aggression or unresponsive)

**Humane Endpoints**

The following criteria should result in euthanasia of the animal:

• An ulcerated tumor, regardless of size and weight.

• Tumor burden exceeding 10% of body weight. (10% typically represents a subcutaneous nodule of 1.5 cm in a 25 gm mouse or 2.5 cm in a 250 gm rat).

• Animals that are moribund or unable to move or failure to respond to gentle stimuli.

• Labored breathing- particularly if accompanied by nasal discharge and/or cyanosis.

• Diarrhea or incontinence.

• Inability to eat and drink.

• Weight loss exceeding 20% of the body weight (the tumor mass should not be included in the calculation).

• If animals are seen to be in distress, regardless of size of the tumor or the weight of the animal.

For animals on studies involving monoclonal antibody production by ascites methods, the recommendations of a National Research Council special committee on monoclonal antibody production should be followed. A copy of the special committee report will be sent to investigators with proposals involving ascites tumors in mice. In addition to the requirements for monitoring and endpoints listed above for all tumors, investigators must provide the IACUC with specific justification for using ascites tumors in mice instead of in vitro culture methods for monoclonal antibody production. Wherever possible, in vitro methods should be adopted as the routine method unless there is a clear reason why their use would present an unreasonable barrier to obtaining the needed product at a cost consistent with the realities of available research funding. In addition to this justification, investigators will be approved for only one tap of ascites as a terminal procedure once sufficient fluid has collected in the abdomen of the animal.
5. Blood collection:

The following guidelines should be adhered to for collection of blood from laboratory animals:

- Animal studies involving blood collection must be approved by the IACUC.
- The maximum allowable volume of blood to be collected at any single bleed for all species must not exceed 10% of the circulating blood volume, when multiple blood collections are a part of the experimental design. Examples are listed in the table below. The following formula should be used:

\[
\text{Body weight (kg) \times circulating blood volume (ml/kg) \times 10\%} = \text{volume of blood allowed for a single bleed. This volume may be repeated after 3-4 weeks.}
\]

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight (Kg)</th>
<th>Circulating Blood Volume (ml/kg)</th>
<th>Recommended Maximum Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse</td>
<td>.030</td>
<td>70 - 80</td>
<td>0.210 - 0.240 ml</td>
</tr>
<tr>
<td>rat</td>
<td>.250</td>
<td>50 - 65</td>
<td>1.25 - 1.63 ml</td>
</tr>
<tr>
<td></td>
<td>.400</td>
<td>50 - 65</td>
<td>2.0 - 2.6 ml</td>
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<td>rabbit</td>
<td>2</td>
<td>57 - 65</td>
<td>11 - 13 ml</td>
</tr>
<tr>
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<td>5.5 - 7.5 ml</td>
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<tr>
<td></td>
<td>60</td>
<td>50 - 70</td>
<td>300 - 420 ml</td>
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The following are normal values for PCV (hematocrit) and total protein in healthy adult animals:

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>PCV (%)</th>
<th>TOTAL PROTEIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse</td>
<td>32-45</td>
<td>6.3-7.8</td>
</tr>
<tr>
<td>rat</td>
<td>35-45</td>
<td>6.3-7.8</td>
</tr>
<tr>
<td>rabbit</td>
<td>30-50</td>
<td>5.3-7.9</td>
</tr>
<tr>
<td>pig</td>
<td>30-50</td>
<td>5.8-7.9</td>
</tr>
<tr>
<td>dog</td>
<td>38-53</td>
<td>5.8-7.9</td>
</tr>
<tr>
<td>nonhuman primate</td>
<td>36-43</td>
<td>6.5-8.7</td>
</tr>
<tr>
<td>guinea pig</td>
<td>37-48</td>
<td>6.3-7.9</td>
</tr>
<tr>
<td>hamster</td>
<td>39-59</td>
<td>6.3-7.8</td>
</tr>
</tbody>
</table>

- When the volume of blood collections proposed are near the upper allowable threshold or when there are other concerns, the IACUC and/or Animal Resources veterinarian may require laboratory monitoring, including measurement of PCV/hematocrit and total protein. The frequency of monitoring required will be determined at the time of protocol review. At the time of each subsequent blood collection, the animal must be monitored for clinical signs of hypovolemic shock and anemia.

  Signs of hypovolemic shock include:  
  - fast, thready pulse  
  - pale, dry mucous membranes  
  - cold skin and extremities  
  - hyperventilation  
  - restlessness  

  Signs of anemia include:  
  - pale ears, footpads (if non-pigmented) and mucous membranes  
  - intolerance to exercise  
  - an increased respiratory rate when at rest

- If records of laboratory values are required by the IACUC, the records should be available for inspection by the veterinary staff at any time. A member of the veterinary staff will review these records while the protocol is active. For rabbits and rodents, information written clearly on the cage card is an acceptable means of recording blood collection dates and volumes as well as PCV/hematocrit and total protein values. For other species, including pigs, dogs and non-human primates, clinical impressions and laboratory values should be recorded in the animal's existing individual chart located in the animal room. An Animal Resources veterinarian must be contacted immediately if any animal shows clinical signs of shock and or anemia, or if laboratory monitoring reveals a drop in PCV and/or total protein of 20% (or greater) of that animal's baseline laboratory values. In such a case, no additional blood may be drawn until the veterinary staff has reviewed and approved further blood collection.
• Collection of blood from rodents via the retro-orbital sinus must be performed using appropriate anesthesia.

• Due to the risks of cardiac tamponade, pulmonary hemorrhage and pneumothorax, intracardiac blood collection is only allowed in the course of a terminal procedure in warm-blooded laboratory species and must be performed only under general anesthesia.

• For larger animals (dogs, pigs, non-human primates), non-mammals or other exotic species, a veterinarian should be consulted regarding the need for necessary and proper sedation/anesthesia prior to collection of blood. For information on appropriate blood collection sites, safe bleeding schedules, or a demonstration of proper and efficient blood collection techniques for laboratory animal species, contact an Animal Resources veterinarian.

6. Pain and/or Discomfort:

In accordance with regulations presented in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act, an integral component of veterinary medical care at a research facility is prevention or alleviation of pain associated with procedural and surgical protocols. Because there is strong scientific evidence that pain and distress are present in animals in comparable situations as they occur in humans, it stands that there is both a moral and legal obligation to prevent or minimize animal pain to the maximum extent consistent with scientific goals. To prevent or minimize pain in animals requires the ability to recognize or better, predict, the need for intervention with analgesic drugs available. With that in mind, the following policy and reference tables (see appendix # I) were created to assist the PI in complying with requirements regarding the alleviation of pain and distress in research animals presented in the animal welfare regulations mentioned above.

Analgesics are required for all procedures likely to cause significant pain in study animals. Analgesics should generally be administered for at least 48 hours following a painful procedure such as a survival surgery. The duration of administration of analgesics should be specified in the protocol synopsis form and approved by the IACUC. The IACUC and attending veterinarian may alter the duration of treatment based on the expected duration of post-procedural pain. In addition, each individual animal should be evaluated at least once daily following a painful procedure, by the investigator or the investigator's staff, for the presence or absence of specific signs of pain as detailed on appendix # I. Recommended analgesic agents, dosages, routes and frequencies are listed on appendix # I. Analgesics are required until specific signs of pain are absent. Although there will be no suitable analgesic that could be administered. It is the responsibility of the investigator to provide to the IACUC a reasoned, scientific justification if analgesics are to be withheld.
7. Transgenic animals:

If transgenic animals will be used on the protocol, this section of the form must be filled out. If the transgenic animals will be produced by another laboratory or institution, the animal procedures used to produce the animals (superovulation, embryo collection, embryo transfer) should not be listed on the protocol form. Instead, the source, which will produce and supply the animals, should be listed. This includes animals produced by the USC transgenic core facility.

Information regarding potential adverse effects and monitoring for adverse outcomes must be included for all protocols in which transgenic animals are to be used. Attachment C of the protocol synopsis form must be completed with this information.

8. Animal Numbers:

Because of the variable nature of animal research at this University we cannot offer specific rules for justification. However, the experimental group size and numbers of experimental groups must be explained and a logical mathematical progression to produce the final total of animals required must be supplied. General guidelines and examples are as follows:

a) If applicable, provide justification for the total number of animals used or produced, not just the number of animals from which data will be collected. In the case of a breeding colony, list the number of breeding animals to be obtained, the total number of offspring born, and the proportion of these actually used for experiments (if all genotypes produced are not usable). For example: “We will introduce 12 breeding animals (6 pairs) of the genetic line into our colony room and produce an estimated 120 mouse pups from the colony. Of these, only 25% will be homozygous recessive to supply the estimated 30 mice for tissue collection as described.”

b) If applicable, include in your justification a consideration of the number of animals that can be expected to be lost to failure of a procedure. For example: “Although we are requesting 8 animals per group, in our experience 20% of the animals will be lost due to improper probe placement. Therefore, we have increased the total number of animals requested from 400 to 500”.

c) Provide justification for the actual number of animals to be used for the experimental procedures.

Valid types of justifications include:

i. **Statistical significance.** Name the type of analysis that will be used and give evidence that the number of animals you are requesting will
provide sufficient statistical power and without using excessive numbers of animals. This evidence can come from personal experience with the species and system, the experience of other investigators, or a power analysis. If the effect size for comparison of experimental groups can be estimated, the IACUC may require the use of power analysis. For example: “Our previous experience with experimental effect sizes in these studies was used to calculate a group size of 8 animals, using an alpha of 0.05 and a beta of 0.10. Since we have 5 groups in each experiment and propose to do ten experiments as detailed in the narrative, we are requesting 400 animals (8x5x10).”

ii. A specific quantity of tissue, antibodies or cells is needed to complete the study. Explain why this quantity is needed and justify the number of animals that will be used to reach this quantity. For example: “For each year of the studies detailed in this proposal, we will require 600mg. of striatal tissue. Each rat striatum weighs approximately 50 mg. Therefore, 12 animals per year are necessary to obtain the needed tissue.”

iii. Pilot study. If you have limited experience with a system such that trial experiments are needed for training or assessment of feasibility, or for any reason you are unable to accurately estimate the number of animals that will be needed to complete the study, you may request a relatively small number of animals for a pilot study. At completion of the pilot study you will be required to submit a separate and final protocol to the IACUC for review in which the total number of animals is adequately justified. For example: “Before performing more detailed studies, we need to determine whether the transgenic mice are producing the protein of interest. To determine this, we must analyze the livers from five transgenic and five control mice. We are requesting only ten mice at this time and will submit a separate protocol form for future studies if the protein of interest is expressed.”

9. Supporting Literature:

Federal law (Animal Welfare Act, 7 U.S.C. 2121, et seq.) requires written investigator assurance “…that alternatives to procedures that may cause more than momentary or slight pain or distress have been considered and written description of the methods and sources used to determine that alternatives are not available.” Therefore, protocols must include this assurance and written description of the methods and sources consulted prior to approval by the IACUC. A search for alternatives to potentially painful procedures must be conducted. Searches should be conducted using databases such as Medline, AWIC, CRIS, Index Medicus, etc. The minimal written narrative should include: the databases searched or other sources consulted, the date of the search and the years covered by the search. At least two
databases must be searched. **A protocol will not be approved without this information.**

10. Signature

The IACUC requires the original signature of the principal investigator for the protocol to be approved. The principal investigator must be a USC faculty member. Signatures of co-investigators and other staff will not be accepted. The signature indicates the person with overall responsibility for the protocol and animals used on the protocol. If a co-investigator will assume this responsibility, the entire protocol should be in their name with their signature.

11. Other IACUC policies

a. Physical Restraint of Animals

Physical restraint is defined by the Guide as the use of manual or mechanical means to limit some or all of an animal’s normal movement for examination, collection of samples, drug administration, therapy, or other experimental manipulation. The IACUC assumes that animals will be restrained for brief periods of time (e.g. a few minutes) for many research applications. However, more prolonged periods of restraint must be listed on the protocol synopsis form and specifically approved by the IACUC.

Guidelines for restraint include the following:

- Restraint devices should not be considered normal housing methods.
- Restraint devices should not be used simply for convenience in handling animals if other safe and handling methods are available.
- The period of restraint must be the minimum required to allow for adaptation and completion of the experimental manipulation.
- Animals placed in restraint devices should be trained and adapted to the equipment and personnel.
- Animals should be observed at appropriate intervals. The IACUC may require continuous monitoring or monitoring at some interval as a stipulation of protocol approval.
- Animals must be removed from the restraint and provided veterinary care if illness or severe behavioral changes occur.
- For some procedures, the use of chemical restraint (e.g. sedation or anesthesia) may be considered more appropriate than physical restraint. Investigators should consult with the Animal Resources veterinary staff regarding sedative and anesthetic agents and methods.
b. Multiple Major Surgical Procedures

A multiple major surgical procedure is defined as two or more major recovery surgical procedures performed on the same animal. This must be specifically justified and approved by the IACUC. Cost is not an adequate reason for performing multiple major survival surgeries. Requests must be made in writing if not included in the original protocol and must include a justification that is based upon scientific necessity.

Frogs of the genus Xenopus are often used for oocyte collection by multiple small incisions in the abdomen. The IACUC has approved a policy allowing up to six survival procedures of this type, with at least 30 days between each procedure in any individual frog.

c. Food or Fluid Restriction

Some experimental situations require food or fluid restriction to reach experimental goals. However, the Guide states that some quantity of food and fluid must be provided for all animals at intervals sufficient to maintain development in young animals and long-term well being of all animals. Overnight food and fluid restriction are automatically approved by the IACUC as part of a standard veterinary care for animals undergoing surgical procedures. The period of restriction for this purpose may also be extended depending on the species and type of surgery to be performed. All other forms of food or fluid restriction must be listed on the protocol synopsis form and specifically approved by the IACUC. This policy applies to studies in which the food or fluid provided is less palatable or causes some physiologic change that results in weight loss or dehydration.

The following guidelines must be followed:

- The degree and period of food or fluid restriction must be the minimum to achieve the desired experimental outcome. The IACUC may require references or other evidence that the restriction is required.
- Behavioral or physiologic indices must be monitored at some interval.
- In the case of food restriction, the IACUC has a general guideline that weight loss should not exceed 20 percent of baseline body weight. Baseline body weight is typically the weight of the animal before restriction began. However, for growing animals such young rodents, baseline body weight should be the weight of control animals fed normal ration ad libitum. Intervals for weighing animals should be listed in the protocol synopsis form and approved by the IACUC.
- For fluid restriction, a more frequent monitoring program for dehydration must be followed. Typically, the IACUC will require monitoring of fluid restricted animals at least once per day. Monitoring
should include daily recording of fluid intake, accurate body weights, or both.

- For conditioned response research protocols, the use of some highly palatable food or fluid as a positive reinforcement should be used instead of food or fluid restriction whenever possible.

d. Induced Seizures in Rodents

Seizures are sometimes induced by pharmacological or other means in rodents as an experimental model. Examples include drug withdrawal, picrotoxin, pilocarpine, and kainic acid treatments of rats or mice to produce animal models for seizures in humans.

The IACUC considers induced seizures to be a category C procedure. Humans with epilepsy do not consider seizures to be painful, and are typically not conscious during a seizure. However, there may be distress related to adverse mental states upon recovery from a seizure in humans. Using the human experience as a guide, animals may also experience some distress (but not pain) on recovery from a seizure or during a mild seizure that does not result in a total loss of consciousness. The level of distress in animals is unknown, but would not be expected to be more than that experienced by animals undergoing other category C procedures.

Single high dose injections of kainic acid (KA) may result in mortality rates of up to 50 percent due to severe uncontrolled seizures. However, one reference (below) indicates that repeated lower dose KA treatment results in a much lower mortality rate with a much higher rate of continued intermittent spontaneous seizures (epilepsy). This model would then result in a more reliable model of the epileptic state with the use of fewer animals.

USC investigators should consider using this method since it represents an improvement based on humane and scientific grounds. The IACUC will send this reference to investigators using KA models in rodents.

e. **Policy on LD<sub>50</sub> Testing in Laboratory Animals**

**Background:**

The classical Lethal Dose 50 (LD<sub>50</sub>) test involves exposure of groups of animals in order to determine acute toxicity of a test drug or chemical. While the purpose of the test is to calculate a single dose that will kill 50 percent of the animals, the test involves groups of animals given various dosages of the drug or chemical. For this reason, certain groups of animals will be given dosages of the test substance that induce a much higher rate of death.

**Available Guidelines:**

While national and international guidelines for acute toxicity testing in animals allow LD<sub>50</sub> testing under certain limited conditions, the use of this test has been strongly discouraged for many years and agencies have worked towards the validation of acceptable alternative toxicity tests that involve more acceptable endpoints. The U.S. Food and Drug Administration requires an assessment of toxicity for new drugs and chemicals, but the LD<sub>50</sub> is no longer specifically required. Similarly, the Organization for Economic Cooperation and Development discourages the use of the LD<sub>50</sub> test and recommends alternatives that reduce numbers and do not use mortality as the endpoint.

**Policy:**

The University of Southern California Institutional Animal Care and Use Committee does not allow the use of LD<sub>50</sub> testing for measuring acute toxicity in animals. Principal investigators that have a demonstrated requirement to study the toxicity of drugs or chemicals in animals will be required to use alternative tests that use fewer numbers of animals and that measure morbidity rather than mortality.

**References:**


United States Food and Drug Administration. Single Dose Acute Toxicity Testing for Pharmaceuticals; Revised Guidance. Federal Register, 61(166).
f. **Policy on Scientific Merit Review of Research Proposals**

**Background:**

The Institutional Animal Care and Use Committee (IACUC) reviews protocols for the use of animals in research and teaching as mandated by Animal Welfare Act regulations and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The primary component of this review is the care and treatment of the animals before, during, and after any experimental manipulation. However, in certain cases, the exact methods must be justified based on scientific necessity, and some components of review may be closely related to the scientific goals of the project.

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

Proposals associated with competitive funding agencies with adequate scientific peer-review processes in place generally will not require additional scientific peer review by the IACUC. However, prior to the peer review process, investigators may wish to conduct pilot studies or studies to obtain preliminary data for application to a competitive funding agency.

Research proposals approved and funded by some external agencies or organizations, or from internal university or departmental funds may have been subjected to little or no scientific peer review.

**Policy:**

The IACUC should be satisfied that all protocols have sufficient scientific merit to justify the use of animals for that study. When a new protocol synopsis form is reviewed by the IACUC, committee members will determine whether there are concerns with regard to scientific merit and may ask for external peer review if the concerns cannot be resolved within the IACUC review process. When a protocol synopsis form is submitted to the IACUC for a three-year renewal and the protocol has not received peer review by an external funding agency during that time, the IACUC may ask the investigator to provide a progress report on the protocol.
Procedures for Scientific Merit Review of New Protocols:

1. If an IACUC member has a concern about the scientific merit of a proposal, the member should request full IACUC review of the protocol synopsis form.
2. IACUC members should not consult other scientists who are not IACUC members until the IACUC votes to seek external peer review and the principal investigator consents to the designated individuals.
3. At the full IACUC meeting, members will discuss the protocol and determine whether additional external peer review is necessary.
4. If the proposal has been submitted to a funding agency that performs peer review, but there are scientific merit questions about the proposal, the IACUC may vote to approve the protocol if there are no issues relating to animal use. In this case, a memorandum will be sent to the investigator with the following wording: “This protocol is approved by the IACUC but work cannot be performed on the proposal until it is peer reviewed and funded by the agency listed on the protocol synopsis form.” If the PI wishes to conduct pilot studies in the interim, the PI may request that the IACUC send the proposal for peer review.
5. If a scientist IACUC member has research expertise in the area of the proposal, that member should be asked to review the protocol synopsis and attend the full IACUC meeting to discuss the protocol. Alternatively, the member may submit comments in writing to the other IACUC members if they cannot attend the meeting.
6. Once the IACUC decides that external peer review is required, one or two reviews of the protocol will be requested. The expert listed on the protocol synopsis form by the investigator may be the reviewer, or other reviewers may be suggested. If other reviewers are suggested, the investigator will be contacted to confirm that the reviewer is acceptable to them. If any additional information is required for the review, that information will be requested from the investigator.
7. A written request will be sent to the reviewer(s) with a requested timeline for review. Reviews should include an analysis of the objectives, hypotheses, methods, and contributions of the project. The IACUC will select reviewers who are knowledgeable scientists and who do not collaborate with the investigator and who do not have a conflict of interest in performing the review. The reviews must be documented in writing and contain sufficient information to support the reviewer’s conclusions.
8. Once the review(s) are completed, the IACUC members will receive copies of the review(s) and make a decision about the approval status of the protocol. If there are remaining scientific merit questions about the proposal that may be corrected by the investigator, the IACUC will withhold approval and submit information to the investigator with the reasons that the protocol was not approved. The investigator may
submit a revised protocol and/or a rebuttal to the findings of the reviewer. If the peer reviewer is willing to be contacted by the investigator, this may be suggested in order to strengthen the proposal for resubmission.

**Procedures for Scientific Merit Review of Continuing Protocols:**

1. At the time of submission of a three-year renewal of a protocol to the IACUC, the principal investigator may be asked to submit a progress report.

2. The progress report must include:

   a. A brief summary of the major findings of the research during the previous approval period.
   b. A description of the specific aims of the research during the next approval period and how these compare with and add to the knowledge gained during the previous approval period.
   c. A description of the numbers of animals used, broken down by species and subproject, if applicable. The Department of Animal Resources can assist investigators by providing total numbers of animals ordered on a protocol.

3. The IACUC will review the progress report along with the renewal protocol form. If an IACUC member has a concern about scientific merit, the protocol will be placed on the agenda for full IACUC review. If the IACUC determines that additional external peer review is necessary, the procedures outlined above for review of new protocols will be followed.
V. Non-Compliance of Investigators to the Institutional Program

Non-compliance with the institutional guidelines and policies has consequences. The IACUC is empowered to suspend any research or teaching project if it finds significant violations. Any deviations from IACUC policies or suspensions of activities are reported to the IO. The IACUC and the IO may also charge scientific misconduct.

For Public Health Service funded research, the IO is required to report serious or continuing noncompliance with the PHS policy, any serious deviations from the provisions of the Guide, and any suspension of a research activity to the PHS Office of Laboratory Animal Welfare (OLAW).

Regardless of funding source, the IO must notify the USDA of any suspensions of activities involving USDA covered animal species. Failure to comply with the Animal Welfare Act can carry penalties that range from reprimands to substantial fines and “cease and desist” orders, in which all activities associated with the use of animals by the offending individual investigator, or by the entire institution, may be suspended. These regulations are not subject to negotiation or individual interpretation by investigators. Thus, it is incumbent upon the prudent investigator to comply with these regulations and encourage colleagues to do the same.
VI. Education and Training Program for Faculty and Staff

A. Purpose

The goal of the USC training and education program is to ensure humane animal care and use by complying with animal welfare regulations, guidelines and institutional policies. This is accomplished by providing training and continuing education programs for all USC scientists, research staff, students, and other personnel involved with animal care, animal use and treatment. The Animal Welfare Act regulations state that training and instruction of personnel must include guidance in the following areas:

a. Humane methods of experimentation and maintenance, including:
   * basic needs of each animal species
   * proper handling, restraint and care of animals used in the facility
   * proper pre-procedural and post-procedural care of research animals
   * aseptic surgical methods and procedures

b. Research methods that limit the use of animals or minimize pain and distress.

c. The proper use of tranquilizers, anesthetics and analgesics for any species of animals used by the facility.

d. Methods to report deficiencies in animal care or other concerns or questions.

e. Provision of information regarding:
   * appropriate methods of animal care and use
   * alternatives to the use of live animals in research
   * prevention of unintended and unnecessary duplication of research involving animals
   * the intent and requirements of the Animal Welfare Act

B. Implementation

The Animal Resources office facilitates the University’s training program. All individuals who work with animals must be trained. Purchase of animals cannot be made until records show that the principal investigator and staff have been trained and it has been documented.

Individualized online training sessions for investigative staff, training classes for animal care staff, seminars and workshops on related topics of animal care and use are the responsibility of the Department of Animal Resources. The Veterinary staff members are available to answer questions and to train or assist anyone who requests help. Training activities are documented. Reference materials including books, videotapes and newsletters on laboratory animals used
at USC are maintained by the Animal Resources Office. These materials are available to all researchers and their staff.

Databases and computerized literature searches are available at the USC Norris Library.

C. Occupational Health and Safety Training

As part of individual online training, all individuals with animal contact receive training in occupational health and safety. This program includes training on individual risk assessment, prevention of exposure to animal allergies, bites, or other hazards, zoonotic diseases (diseases potentially transmissible between animals and humans), and mechanisms to report problems for follow up as needed. The main objectives of the training are to instruct individuals on hazard recognition and avoidance. In particular, information is presented on how to prevent the production of allergies. As part of the training, each individual is informed about the medical surveillance questionnaire process to enroll in the USC occupational health program for animal handlers.

It is the responsibility of the Safety Office to ensure all employees are aware of general hazards in the workplace when animals are involved. It is the responsibility of the principal investigator to ensure that all employees are aware of the specific hazards associated with their job duties, and that safe practices are used daily. The hazard, risk, and preventive measures associated with a specific research project should be understood by all employees. Depending on the species of the animal, the degree of exposure, or an individual's prior sensitivity, not all employees will be affected equally. An important aspect of working safely is thorough communication between the animal care staff, research staff, and safety staff.

Individuals who intend to work with nonhuman primates must attend additional training sessions relating to additional risks associated with these species. In particular, macaque monkeys may harbor cercopithecid herpesvirus 1 (herpes B virus). The risks and preventive procedures for this viral infection must be understood by all staff who work with these species. Also, there are specific procedures for medical management of possible exposures to the virus through bites, scratches, or mucocutaneous contact. Animal Resources veterinary staff provides this additional training.
VII. General Animal Resources Policies

The USC Animal Resources Office of the Director is located on the Health Sciences Campus, Hoffman Bldg. (HMR) room 214. The Department of Animal Resources is responsible for the husbandry and veterinary care for all vertebrate animals maintained for research and teaching on the Health Sciences and University Park campuses.

A. Assignments and Use of Animal Rooms

Animals are housed by species and health status to minimize infectious disease problems. Investigators are encouraged to notify the Director’s office in the Department of Animal Resources on anticipated needs as far in advance as possible. Use of animal facilities for purposes other than experimental animal care and use is not allowed.

B. Housing Animals in Laboratory Areas

Experimental animals must be housed in designated animal holding facilities and may not be kept outside these areas for more than 12 hours or overnight without specific approval from the Animal Resources and variance approved by the IACUC.

C. Access to Animal Facilities

All investigators with approved animal use protocols must submit a list of individuals who will be handling or otherwise exposed to animals. This list, which should include the approved protocol number, shall be submitted to the Animal Resources Director’s office. University identification numbers must be provided for the USC Security List. This list is maintained with strict confidentiality. The submitted names should correspond to the names mentioned/listed on the approved protocol. No individual will be allowed access to the animal facility without proper training. To protect USC employees, students and the general public from unnecessary exposure to potential hazards and to protect animals and research from interference from unauthorized personnel, the designated public elevators should be used. Both faculty and staff are requested to assist in enforcing this policy and to report exceptions to the Security Office at (323) 442-1200 for HSC and (213) 740-6000 for UPC.

Tours of USC animal facilities need to be arranged in advance by contacting the Director’s office. Approved visitors must be properly escorted throughout the facilities and adhere to the protective clothing requirements in all areas. Cameras and video equipment are only allowed with prior permission. Children are not allowed in any animal facilities at USC.
D. Eating and Drinking in Animal Facilities

Eating and drinking are prohibited in all animal facilities and elevators except in specifically designated areas (e.g. lounge areas).

E. Noise in Animal Facilities

All personnel should be aware that excessive sound in animal research facilities must be avoided. Activities such as moving cages or equipment must be performed as quietly as possible. Radios, alarms, and other sound generating devices are not allowed in animal facility rooms or hallways directly outside of animal rooms unless they are part of an IACUC approved protocol or an enrichment program. This includes small personal music players or similar devices even if headphones are used. These devices may be used only in areas not housing animals (such as break rooms) or otherwise must have the approval of the Director of Animal Resources to be used.

F. Use of Ether in Animal Facilities

The use of ether or similar explosive, flammable agents is not allowed in any animal holding facility. The Animal Resources veterinary staff can supply alternatives to the use of ether as an inhalation anesthetic. The use of ether under any condition requires clearance from the Safety and Risk Management department.

G. Pets

Because of the risk of transmission of infectious diseases and the numerous regulations with which USC must comply, pets are not allowed in any animal facility.

H. Emergencies

The Animal Resources emergency plan coordinates with overall emergency plans for the Health Sciences Campus and University Park Campus. In the case of an emergency such as fire, or earthquake, it is important to consider animal safety, but to prioritize human safety in all cases. Therefore, in an emergency of sufficient intensity to require building evacuation, animals should be left in the facility and all human occupants should evacuate immediately. Once the immediate threat has subsided, personnel from the Safety Office and Animal Resources will determine the status of all animals and make any decisions regarding animal evacuation if necessary.
VIII. Procurement of Research Animals

Federal regulations (e.g. Animal Welfare Act) and granting agencies (e.g. NIH), require complete recording and reporting of animal orders and/or acquisitions. Purchases of all research animals must be placed through the USC Animal Resources Business office, Hoffman (HMR) room 215. Procurement of animals will be processed and acquired only if there is a file of an existing, fully approved animal use protocol and that all training requirements have been met. Purchase of non-human primates requires a permit from the California Department of Health Services. In addition, purchase of some animals, such as frogs and ferrets, require a permit from the California Department of Fish and Game.

The USC Animal Resources Business office uses several criteria to identify appropriate vendors to fill animal orders. These are as follows:

A. Account Maintenance

Prior to any receipt of animals for research an account (either an Internal Requisition or external-source Purchase Order) must be established with the Animal Resources Business Office. This ‘account’ should include sufficient funding for the duration of the project or a yearly amount of award. Internal Requisitions refer to a valid USC 10-digit account number and reference the investigator's name and IACUC-approved protocol for each account. A monthly statement is then sent informing the investigator of all activity on each Animal Resources account (Requisition or Purchase Order number) with a balance of funds and animals shown for the preceding month. For services to be rendered such as animal purchases or housing of animals the Animal Resources account must be in good standing. Any notification of delinquent amounts should be satisfied quickly to avoid delays in purchases or services. All financial questions should be satisfied quickly to avoid delays in purchases or services. All financial questions should be directed to the Animal Resources Business Office.

B. Licensing and certification of animal health.

The University requires vendors to provide health status reports of their animal colonies. Animal Resources conducts health surveillance testing on an ongoing basis. Investigators may request a specific vendor; however, Animal Resources reserves the right to confirm the animal health status and approve or disapprove requests accordingly.

C. Transportation practices.

Animal Resources staff inspects arriving animals and will reject any filtered containers that have been compromised. Vendors are required to replace animals that have arrived in damaged filter containers or are sick, injured or do not meet other purchase order specifications. Animal Resources uses only vendors who
transport animals using trained personnel and vehicles or carriers, which meet federal requirements.

D. Service practices.

Suppliers must be willing to meet necessary delivery schedules and to notify the buyer whenever there is a possibility of contamination in their facilities.

To prevent the chance of introducing disease into the animal facilities at both the HSC and UPC campuses, all arrangements for acquiring and housing live vertebrates must be made through the Animal Resources Office. The Animal Resources Business office will assist investigators in the acquisition of the required permits.

The Animal Resources Business office requires a V1 form to process animal orders. The V1 form must be received in the business office at least 3 working days prior to requested delivery date for rabbits and rodents. Dogs, pigs, non-human primates and ferrets generally take 2-3 weeks from order to delivery due to other factors (e.g. availability of vendor, vendor location, shipping, permits, etc.). All legal holidays regulate this schedule accordingly.

Delivery dates are scheduled on Tuesdays and Thursdays to prevent problems associated with arrival on weekends and holidays. Special accommodations can be arranged.
IX. Animal Care

A. Animal Care Schedule

The animal care staff performs animal care procedures 7 days a week, 365 days per year.

B. Animal Care Provided by Investigator's Staff

Under certain circumstances, investigators may need to provide certain aspects of daily care of experimental animals to accomplish specific scientific goals. In such instances, the Director of the Animal Resources Program and IACUC must approve animal care provided by investigator staff and may then accommodate these requirements within the operational framework of the program and facilities.

C. Animal caging:

The Animal Resources office is responsible for determining the proper cage size for laboratory animals. This is to ensure that housing practices meet the NIH standards and the Animal Welfare requirements and at the same time meet the needs of the research. Special housing requests and exceptions should be noted in the animal use protocol, with justification for the IACUC and attending veterinarian’s approval. The request must be in writing.

USC utilizes filter top cages for the housing of transgenic and immune compromised animals to reduce the risk of disease contamination in rodent colonies. Proper training is required for individuals who are unfamiliar with the use of these cages. Investigators are encouraged to contact appropriate animal care facility supervisors with any questions.

D. Environmental factors

1. Ventilation

Heating, ventilation, and air conditioning in animal facilities requires constant monitoring to assure proper airflow and appropriate temperature and humidity levels. The Animal Resources animal facility supervisors and University maintenance personnel ensure that the environmental systems in the animal facilities are in compliance and are functioning properly. Any departures from the required levels should be reported to the Director of the Animal Resources. Emergency facility problems encountered after working hours (6:00 AM - 5:00 PM) should be reported to the Department of Public Safety. They will contact the appropriate individuals to respond to and correct the problem.
2. **Illumination**

Special light cycles in animal housing areas can be arranged through the Animal Resources Director’s office. Regular diurnal light cycles are provided by a time-controlled light system. The animal care facility supervisors and USC maintenance personnel check the systems daily. The Animal Resources staff is responsible for establishing and maintaining light cycles in the animal housing areas on both campuses.

3. **Temperatures and Humidity**

Requirements for temperature and humidity in the laboratory animal facility are clearly defined and carefully monitored on a daily basis to ensure stability. The Laboratory Animal Technicians are trained to check, record and report to their supervisor any deviations in environmental requirements.

E. **Feed and Water**

The Animal Resources is responsible for providing adequate and appropriate food and water. Special research diets and departures from required feeding and watering schedules must be documented in the animal use protocol for the IACUC’s approval. Facilities housing dogs, pigs, rabbits and some rodents contain automated watering systems. For transgenic colonies and other rodents, sterilized or purified water is provided in bottles.

Any special requirements (e.g. drugs) must be requested to the animal facility supervisor. Requests must be in writing and should include type, duration of administration of each special substance.

F. **Sanitation of Cages**

The NIH Guide and the Animal Welfare Act have established guidelines for the frequency of the cleaning of animal rooms and the changing and cleaning of cages. Animal Resources personnel follow Standard Operating Procedures and schedules regarding sanitation to ensure compliance. Cages or animal carriers that are taken to the research laboratories for acute rodent procedures should be returned to the wash room or to any animal care technician or supervisor.

G. **Disposal of Animals**

Animal housing facilities have designated freezers and refrigerators for animal carcass disposal and incineration. No food, drugs, supplies or other materials should be placed in these freezers or refrigerators. Radioactive and biohazardous animal carcasses, waste, and bedding must be disposed of according to the procedures established and administered by the USC Safety office. The safety office and the appropriate safety committee must approve radioactive, hazardous and infectious substances when used on an animal protocol.
H. Proper identification of animals

Proper maintenance of records and appropriate animal identification are required.

Cage card identification must have the following information:

* Principal Investigator (PI)
* Protocol number
* Species
* Breed and Strain
* P. I. or contact person phone number
* Contact Person
* Pertinent dates (e.g. animal DOB)
* Request number (V1 number)
* Requisition or PO number

I. Overcrowding

Overcrowding is addressed in federal regulations and is not allowed. In studies involving breeding, weaning of litters is the responsibility of the researchers or their staff and requires proper identification. Animal Resources animal care technicians will report any overcrowding to the principal investigator and/or their staff members. If not corrected within 24 hours, the overcrowding is reported to the Animal Resources Director’s office and the investigator will again be contacted. Within 24 hours of this report, Animal Resources will separate the cages and charge the investigator for this service. Repeated occurrences may be reported to the IACUC.

J. Individual Housing of Animals

The Guide states that consideration should be given to an animal's social needs. The social environment usually involves physical contact and communication among conspecifics, although it can also include noncontact communication through visual, auditory, and olfactory signals. Whenever appropriate and possible, social animals should be housed in physical contact with conspecifics (pair or group housing). However, individual housing is allowed under the following circumstances:
1. When group housing is not possible for experimental reasons.

Examples include:

- Animals with surgical incisions, catheters or other devices that might be damaged by a conspecific and result in harm to an animal.
- Animals on behavioral protocols where the presence of a conspecific might alter the experimental results.

2. When the behavior of the animals in a group may result in harm to one or more animals.

Examples include:

- Large animals such as dogs, swine, or nonhuman primates that are observed to be aggressive towards one another.
- Animals such as male mice that are known to fight when placed in groups.
- Animals such as mice with certain genetic traits that make them more aggressive and likely to fight with conspecifics.

3. When group housing is not possible for veterinary reason, such as quarantine of an individual animal with an infectious disease.

If single housing of animals is requested by an investigator, the IACUC may approve the request based on justification for experimental reasons. Single housing of animals for other reasons (behavioral, veterinary) may be approved by the Animal Resources veterinarian without specific justification from the investigator.

K. Per Diem Charges

The per diem charges cover boarding, feeding, watering, cage and room cleaning, sanitation and animal incineration or disposal. The charge does not cover veterinary, diagnostic laboratory, pathology, and services for medical problems, emergency treatments resulting from experimental manipulations, special diets, special transportation, or special husbandry requests. These services will be billed to the investigators account. Per diem rates are established and updated annually. The rates are subject to change based upon ongoing cost analysis.

NIH accepts charges for the acquisition, care, and use of experimental animals as allowable costs as part of budgetary requests in submitted grant proposals in accordance with guidelines found in the publication entitled Cost Analysis and Rate Setting Manual for Animal Resource Facilities (NIH publication no. 80-2006). Copies are available in the office of the Director, Animal Resources Program. For information regarding specific per diem rates, please contact the Animal Resources Business Office.
Animal care technicians record the animal inventory for each room. Per diem charges for animal care begin on the day the animal arrives and is caged in the animal room. For animals born in the animal facility, per diem charges begin on the day of weaning (typically at 21 days of age for rats and mice). Investigator staff should inform the technicians of any changes in the numbers of animals housed. White boards are located in facilities for this purpose.

L. Animal Transfers and Shipments

The Animal Resources veterinary staff must approve all animal transfers and shipments. This is to insure that the health of animals is protected during and after the transfer. In addition, all animals housed for teaching or research must be accounted to a protocol that has been approved by the IACUC. The following are guidelines for animal transfers and shipments:

1. Animal Transfers within USC facilities:

   To request a transfer of one or more animals from 1) one PI to another within the USC campuses; 2) one facility to another within the USC campuses; or 3) from one IACUC-approved protocol to another IACUC-approved protocol, investigators are asked to fill out Animal Transfer Form A: Request for Animal Transfer within USC Facilities. This form is available in the Animal Resources office. Additional forms are available in the Animal Resources Director's office. The Animal Resources Director's office will approve the transfer and Animal Resources staff will move the animals to the new location. Transfer of animals requires that the animals have not been used for a previous study and that the investigator receiving the animals is approved by the IACUC for their use. Care should be taken in movement of animals between laboratories and animal housing areas to reduce stress the animal may experience while being moved. All research animals should be transported in approved cages and carriers that can be sanitized after each transport and must be of sufficient design to prevent escape and injury. Animals are not to be carried by hand or in open boxes. Carriers should be covered with a towel or drape during transportation so the animals cannot be seen and the exposure of personnel to odors and allergens is minimized. All carriers and cages should be returned to the cage washing room area. Please contact the Animal Facility supervisor or the Director’s office for selection of proper containers. Requests for transportation by Animal Resources are subject to a service charge and should occur at least 24 hours in advance Monday though Friday.

2. Animal transfers to non-USC facilities:

   Shipment of animals is covered by Federal, State and University regulations and guidelines. Compliance with these regulations generally requires that a licensed, accredited veterinarian examine and certify the health of the animals
prior to shipment. Therefore, no animals will be allowed to enter or leave USC campuses without prior approval of the Animal Resources veterinary staff. Regulations and guidelines require that veterinarians examine and issue a health certificate prior to shipment. Veterinarians are knowledgeable concerning current shipping requirements and are qualified to sign federal health certificates. They will assist investigators in such matters, but they must be notified well in advance of the shipping date.

In the case of shipping animals to non-USC facilities, the policy is to communicate with the receiving facility to confirm that the individuals have an approved animal use protocol and the receiving veterinarian is aware of the animal shipment and their health status. In order to facilitate this communication, investigators are asked to fill out Animal Transfer Form B: Request for Import/Export of animals. A copy of this form is available in the Animal Resources Business office. Please see the standard operating procedures on animal transfers available in the Animal Resources Business office. Questions regarding animal shipments should be directed to the Animal Resources Business office at 442-1695. This office maintains a listing of approved carriers for animals.

It is the responsibility of the investigator to clearly identify the animals to be packed and shipped by Animal Resources staff.

3. Animal transfer from non-USC facilities:

Similar practices are applied when animals are received from a source other than a commercial vendor (e.g. an investigator in another institution). To facilitate this transfer, investigators are asked to fill out Animal Transfer Form B: Request for Import/Export of Animals. A copy of this form is available in the Animal Resources office. The information found on this form will be used to acquire health information for the animals being shipped to USC. A USC veterinarian will review this information and contact the USC investigator and the originating facility when the transfer has been approved or declined. Depending on the species and health status of the animals to be received, a quarantine period may be required upon arrival at USC.

Regarding quarantine, please note: All mice transferred to USC are subject to a 6 week quarantine period. The quarantine room is open for a short time during the first 2 weeks of each month. A quarantine calendar with information regarding open dates, treatments, and testing and release dates is available on the Animal Resources and IACUC website and is updated regularly. Information on quarantine standard operating procedures, including treatments and diagnostic testing, is also available on the website for review. The PI and PI’s staff are generally not allowed access to the quarantine room and are discouraged from planning any procedures which involve direct contact with the imported mice during the quarantine period. Any special requests by the PI which may require a deviation from standard operating
procedures for quarantine mice should be requested from and approved by a
Department of Animal Resources (DAR) veterinarian before the end of the quarantine period only for transport to the PI’s laboratory to be
used immediately for terminal procedures. Rodents are not allowed back into
the quarantine room once they have been removed. Any pairing (breeding)
requests from the PI should be arranged with the technician or veterinarian
(the animal care technician or veterinarian will place together the breeding
pairs designated by the PI). USC colony mice with a clean health history can
be transferred to the quarantine room for breeding with newly imported
animals only during the open quarantine period for that group (requires an
Animal Transfer Form A to be approved by a DAR veterinarian). PIs are
responsible for all costs of the import which may include shipping costs,
quarantine fees and any special services required. Please contact the DAR
Business Office at (323) 442-1695 for a current estimate of rodent import and
quarantine costs.
X. Veterinary Medical Care

A. Preventive Medicine

1. Purchase of animals

Newly arrived animals can introduce disease into established colonies. The Veterinary diagnostic laboratory and clinical veterinarians will monitor animal health status in addition to the health information supplied by the approved supplier.

2. Quarantine and Stabilization

Animal Resources veterinarians may require quarantine of animals under certain circumstances. For example, groups of animals that are diagnosed with an infectious disease may be separated and quarantine until the problem can be corrected. The length of the quarantine period is determined by factors such as species, vendor source, and procedures to be performed. In addition, quarantine of nonhuman primates for 30 days is required by state regulations. Newly arrived animals regardless of source should be allowed time to adapt to new conditions. For acute experimentation with requirements for physiological data, 72 hours acclimation period is recommended. For chronic projects, 1-week acclimation is recommended.

3. Separation of Animal Species and Study

Separation of animal species by room is recommended to reduce the chance of disease transmission and to avoid inter-species aggression or distress. This is accomplished by housing animal species in separate rooms. In addition, groups of animals may be separated for experimental reasons. For example, animals on studies involving biohazards will normally be maintained separate from other animals.

I. Diagnosis, Treatment and Disease Control

1. Routine Veterinary Care

The Animal Resources care personnel check the animals daily, including holidays and weekends, for signs of injury, illness or abnormal behavior. In cases where such observations will interfere with experimental goals, arrangements must be made to ensure that there is an adequate monitoring system.

A health surveillance program is in effect at USC for each species. In-house health monitoring is done for rodents as part of the sentinel program, in which samples are periodically collected for serology, bacteriology, parasitology and
pathology. An in-house diagnostic laboratory performs the analyses, and investigators are notified of the problems that may affect their animals. Following release from quarantine, non-human primates are tested two times a year for evidence of communicable enteric bacteria, parasites and tuberculosis. Testing also includes routine physical examination and CBC.

2. Emergency Care

Any health problems noted by research staff, Animal Resources animal care technicians at any time, including weekends, holidays and evenings must be reported to Animal Resources veterinarians.

A list of the phone and pager numbers for Animal Resources veterinarians is posted outside individual animal rooms and facilities.

C. Anesthesia and Analgesia

1. Policies

All animal policies and programs are reviewed by the IACUC to ensure that proposed anesthetics and or analgesics are appropriate for the species and research goals. Veterinarians are available to provide assistance with or training in proper administration and use of anesthetics, analgesics and tranquilizers.

Documentation is required of all surgical procedures including the specific procedures to be performed, the timing of the administration of anesthetic, analgesic or tranquilizers and anesthetic parameters (heart rate, BP, respiration, temperature, etc.) to be monitored. All records are subject to inspection during bi-annual inspections by the IACUC, veterinarians and USDA inspectors. Information concerning volume of blood collected, frequency of blood withdrawal, and duration of pre- or post-operative fasting should be included in an individual animal record or experimental notes. During the annual protocol renewal, animal records will be reviewed by veterinarians and non-compliance will be reported to the IACUC.

2. Guidelines for the use of Tribromoethanol (Avertin) Anesthesia in Mice

Background:

Tribromoethanol, formerly available under the trade name Avertin®, has been widely used in rodents as a surgical anesthetic for procedures such as embryo transfer, vasectomy, and tail amputation for Southern blot analysis. The rapid induction, adequate plane of anesthesia, and lack of complications make this anesthetic effective and simple to use.
However, there have been several reports of low margin safety and serious side effects which have, in the past, prompted veterinarians to discourage its use. Reported side effects include postoperative fatalities, associated with peritoneal fibrous adhesions and high mortality after administration of a second anesthetic dose, regardless of the interval between doses. These claims were not substantiated. Furthermore, more recent reports failed to confirm the reported side effects, but instead, illustrated the advantages of tribromoethanol anesthesia. It is now generally accepted that variable results produced with tribromoethanol, including the reported side effects, are due to decomposition products in stock solutions of the anesthetic.

Given the positive reviews tribromoethanol has received in recent literature combined with reports by campus investigators at USC claiming years of successful use, it would appear that tribromoethanol is a safe and effective anesthetic. As there are no acceptable alternatives at this time, we are advocating the use of tribromoethanol as an anesthetic for use in short surgical procedures in mice with the stipulation that 1) the anesthetic be made according to USC guidelines (which are based on procedures previously published) and 2) precautions are taken to prevent its decomposition, as described below.

**USC Guidelines:**

**Formula for tribromoethanol Anesthesia**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2,2-Tribromoethanol</td>
<td>2.5g</td>
</tr>
<tr>
<td>2-Methyl-2-butanol (tertiary amyl alcohol)</td>
<td>5 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

**Method:**

1. Add Tribromoethanol to butanol and dissolve by gently heating (approximately 50º C) and stirring.
2. Add distilled water and continue to stir until butanol is totally dispersed.
3. Sterile filter through a 0.22 micron filter.
4. Aliquot into storage containers. Solutions should be maintained in either brown glass or foil covered bottles and refrigerated when not in use. Failure to protect tribromoethanol solutions from light or to store them at 4º C will result in the formation of irritant decomposition products that can cause peritonitis and death.
5. Warm to 37º C and shake well before use.
6. The following procedure can be added to test for decomposition products before use: provided the pH of the original solution was >5, decomposition products can be revealed by adding 1 drop of Congo Red (0.1% w/v) to 5ml anesthetic. Purple color developing at pH <5 indicates decomposition to dibromoacetic aldehyde and hydrobromic acid. If this occurs, the anesthetic should be discarded, as it is
toxic and can cause death within 24 hours of injection. Solutions should be tested once per month during storage.

**Dosage:** The dose for IP administration to mice is 240 mg/kg. This agent usually produces about ten to fifteen minutes of surgical anesthesia in mice. Full recovery takes about 90 minutes.

**D. Surgery and Post Operative Care - General Policies**

Research involving animal surgery must be performed with attention to pre-surgical planning, personnel training, surgical technique, and animal physiologic states.

1. **Definitions:**

   **Survival surgery** is defined as any surgery from which the animal recovers from the effects of general anesthesia.

   **Non-survival surgery** is a surgical procedure performed under general anesthesia at the conclusion of which the animal is euthanized without regaining consciousness.

   **Major Surgery** is defined as any surgical manipulation or intervention that penetrates or exposes a body cavity or has the potential for producing a substantial physical or physiologic impairment of physiologic effects in an animal that is expected to recover.

   **Minor Surgery** is any surgical procedure in which skin or mucous membrane is incised (e.g., vascular cutdown for catheter placement or subcutaneous implantation of pumps). Minor surgical procedures are often performed under less stringent conditions, but still require aseptic technique.

   **Multiple Major Survival Surgery** is defined as two or more major recovery surgical procedures performed on the same animal. This must be specifically justified and approved by the IACUC (see section IV).

**E. Survival Surgery in Rodents**

The federal *Animal Welfare Act* and the NIH *Guide for the Care and Use of Laboratory Animals* both have separate requirements for rodent (rats, mice, guinea pigs, and hamsters) vs. non-rodent mammal procedures. For example, while dedicated surgical facilities are required for all non-rodent procedures, they are not required for rodent survival surgery. However aseptic technique is required for all species. Useful suggestions for applying aseptic technique to rodent surgery in an acceptable and efficient manner have been published (Cunliffe-Beamer, T.L., AWIC Newsletter, Vol. 4, No. 2, 1993). The following
guidelines were written bearing in mind that modification of standard aseptic surgical technique is acceptable for rodents, as long as it does not compromise the well being of the animal.

1. Surgical facilities:
   a. Rodent surgery can be performed in any room or portion of a room that is easily disinfected. This would include, but is not limited to, a clean, uncluttered lab bench, table, or in a laminar flow, HEPA-filtered hood.
   b. The dedicated surgical area should not be used for any other purposes during the time of surgery.
   c. Prior to and between surgeries, the surface upon which surgery will be performed must be cleaned and properly disinfected (quaternary ammonium disinfectants or 70% alcohol are good choices).

2. Surgical Instruments:
   a. Surgical and other instruments must be sterilized for use in rodent survival surgery. Steam, dry heat, gas (ethylene oxide), chemical sterilants (e.g. glutaraldehyde, used in accordance with USC Safety Department guidelines) and radiation can be used to sterilize instruments.
   b. Decontaminating instruments between surgeries when performing surgery on multiple animals is required. Wiping tips of instruments with 70% alcohol between animals is not acceptable. If repeated use of surgical instruments is anticipated, a dry bead sterilizer is recommended as the most safe and convenient option. Animal Resources veterinary staff members can provide information on alternatives.

3. Animal preparation:
   a. Pre-surgical monitoring is to include a visual examination of the animal. If any animal appears sick (e.g. lethargic, ruffled or discolored hair coat, hunched posture) consult with an Animal Resources staff veterinarian before proceeding with the surgery.
   b. Aseptic technique includes preparation of the animal, such as hair removal and disinfection of the operative site (e.g. surgical iodine (betadine) and/or 70% alcohol are acceptable).

4. Surgeon Preparation:
   a. Any individual performing surgery on rodents must have documented training by an Animal Resources staff veterinarian on file.
   b. Modified aseptic technique is acceptable but must include the provision of decontaminated surgical attire (e.g. a clean lab coat or surgical scrubs), as well as wearing a mask and sterile surgical gloves (clean latex examination gloves swabbed with 70% alcohol are an acceptable alternative to sterile gloves if using a glass bead sterilizer for instruments).
5. During Surgery:

   a. The surgical field must be kept as sterile as possible throughout the procedure to reduce the likelihood of infection.
   b. Monitor the animal carefully during the surgical procedure. Surgeons should pay close attention to the animal's heart rate, respiratory rate, and body temperature.

6. Postoperative care:

   a. In the immediate post-operative period, prevent hypothermia by placing the animals in a warm room or cage. Supplemental warmth may be supplied by a heat lamp (located at least 2-3 feet from the animal) or warm water bottle or bags located next to the animal. If heating pads are used, they should be insulated from the animal using towels or drapes so the animal does not overheat. Monitor for dehydration. Dehydration can be ameliorated by the administration of appropriate fluid therapy.
   b. To prevent cannibalism of suffocation, house rodents individually until they are ambulatory. Animals should not be returned to the animal facility until they are sternal and clearly beginning to wake up.
   c. External sutures or wound clips should be removed 7-14 days after surgery.
   d. Post-surgical animals should be seen every day by a member of the investigator's staff or other individual to whom post-operative care has been delegated. Animals should be observed daily until all sutures, wound clips, or other implanted devices have been removed.

7. Records:

   a. A peri-operative record should be kept in the laboratory.
   b. A composite record may be used for a group of rodents. Such a record would include a list of animal numbers, surgery days, a notation that the animals have been checked, any abnormal observations, and a list of any anesthetics, analgesics and other therapeutics given including drug names, dates of use, doses, and routes of administration.
   c. Records should be kept current during the immediate post-operative period (e.g. 7-14 days or until all wounds have healed and all sutures/wound clips have been removed) and should include dates for study completion and animal euthanasia.

F. Survival Surgery in Animals other than Rodents

1. Facilities:

   Both minor and major survival surgical procedures must be performed in a dedicated surgical facility, which is approved, by the attending veterinarian and IACUC.
2. Procedures:

Strict aseptic technique must be used for all surgical procedures. All personnel must wear sterile surgical gloves, gown, cap, and mask. Sterile surgical instruments and supplies are required, as well as maintenance of an aseptically prepared surgical field.

3. Pre-operative and post-operative care:

Animals should be fasted prior to anesthesia and surgery to prevent vomiting and aspiration and problems associated with abdominal distension. Pre-surgical evaluations should be performed and baseline physiologic parameters such as body temperature, respiration, and heart rate should be recorded before anesthetic administration.

Body weight should be measured for proper drug dosages.

Post-surgical analgesics should be administered routinely and clinical observations of the animal should be recorded. Intensive care units are available in the Hoffman surgical area for the sole purpose of housing recovering anesthetized animals.

The use of post-surgical treatments, including analgesics, fluids and antibiotics should be documented and animals should be monitored. Observations of animal condition, abnormal behavior, pain, appetite, excretions, body temperature, respiration and heart rate are also to be recorded and should include the observer’s initials, date and time the observation was made. Surgical incisions are to be checked daily until surgical sutures are removed.

G. Euthanasia

Euthanasia is generally performed when animals experience severe or chronic pain or distress that cannot be relieved, or at the end of the experimental procedure or project. The method of euthanasia must be described in the animal protocol and approved by the IACUC and must be consistent with the recommendations of the AVMA Panel on Euthanasia. Euthanasia should be performed quickly and effectively in a non-public area. In addition, the training of the personnel performing the euthanasia is critical. If any staff member needs assistance with the euthanasia, they should contact one of the Animal Resources veterinarians who will provide assistance at no charge. Rodents are commonly euthanized using carbon dioxide. However, carbon dioxide gas must be provided from a tank source (not dry ice) and a follow-up method such as cervical dislocation must be employed.
XI. Occupational Safety and Health

A. Introduction

An occupational health program must be part of the overall animal care and use program. To ensure its effectiveness, it will rely on administrative support and interaction among all parties involved in environmental health and safety. Operational and day-to-day responsibility for safety resides with the principal investigator in the laboratory, and the animal care supervisor in the animal room, and depends on the performance of safe work practices by all employees. This program is mandatory for all personnel working with animals in the laboratory or in the animal facilities. According to the category of exposure, a medical surveillance program will be designated for every employee. All personnel must participate in an educational and training program to ensure a specific level of knowledge in zoonoses, personal hygiene, the use of personal protective equipment, equipment performance, precautions to take in unique situations, cause and control of allergies, and appropriate exposure follow-up procedures.

B. Risk Assessment

There are two distinct areas of hazard to assess when working with animals in a research facility. First, there is the risk of allergies and zoonotic disease. Second, the research may involve hazardous chemical, biological or radioactive materials. Personnel should assess the dangers associated with these materials and animals, and select the appropriate safeguards. Other considerations should be the exposure intensity, duration and frequency, and susceptibility of the personnel based on their occupational history. All animal research involving infectious agents, human tumor cells, recombinant DNA, hazardous chemicals, radiation, or the use of animals that present other unique hazards will be reviewed and approved by the IACUC and the appropriate safety committee.

C. Exposure Control Methods

Exposures to occupational hazards are controlled through the application of engineering controls, work practices, and the use of personal protective equipment. The types of hazards of concern are direct skin contact, inhalation, and contact with bedding waste, and bites. The use of chemicals, radiation or biological agents will present an additional hazard specific to the agent.

1. Engineering Controls

Procedures involving some chemicals or radioactive materials are often performed in the fume hood. Fume hoods are used to exhaust toxic, offensive and chemical fumes from the work area, and should be certified annually.

Procedures involving volatile gas anesthetics must be performed under conditions that ventilate the anesthetic gases away from personnel. Gas
anesthesia may be safely performed in a fume hood. Ether is not allowed for rodent anesthesia.

Procedures involving biological agents may be performed in a biological safety cabinet. These cabinets are primary containment devices for work with infectious agents. Biological safety cabinets must be certified when installed, relocated, and annually. A nonstationary cabinet must be certified every time it is relocated.

When an infectious agent is to be injected into, or fluid aspirated from, an animal, a "luer-lock" syringe or an integral needle and syringe unit must be used. Needles must never be recapped using two hands. The needle and syringe must be dropped, without recapping, into a sharps container that is immediately accessible for use.

The facilities must be adequate for the safe conduct of research (refer to the Biomedical and Microbiological Laboratory guidelines), and the staff competent in handling biohazardous materials.

The fume hood and the biosafety cabinet should be located away from doors, supply air ducts, and high traffic areas. The face velocity for fume hoods should be around 100 lfm (USC policy for fume hoods is a range of 125-150 lfm) to prevent turbulence at the hood or inadequate airflow. Biological safety cabinets must be recertified annually. The USC Safety Office can supply information on certification.

Cage filter tops are used to prevent cross contamination with infectious agents between and among animals and people by preventing particles from entering the cage. In most facilities, cages should be placed inside a biosafety cabinet or change station when the top is removed.

Room ventilation should maintain the temperature and humidity at a comfortable level. The airflow should be directional to prevent contaminants from going into unprotected space.

2. **Work Practices:**

Employees should know the hazards associated with the procedures that they are performing, the route of transmission, and the practices that will minimize exposures.

**Handling and Transport of Animals:** Handle animals with care and proper restraint to prevent the generation of aerosols, skin contact, scratches and bites. Wear personal protective equipment specific for the exposure related to the animal being handled or transported. Aerosols from litter remain airborne for 15-35 minutes. The protein in saliva and urine is allergenic; therefore, proper hygiene is required to reduce the allergens.
a. **Personal Hygiene:** A sink, soap and towels must be available in every lab and animal room. Hands should be washed before and after handling the animals and whenever gloves are removed. Paperwork should be handled only after gloves are removed and hands are washed. There should be no eating, drinking, smoking, application of cosmetics or lotions, or other activities that can increase the risk of exposure. Animal allergens stick to gloves and hands and will, therefore, adhere to inanimate objects. The next person to touch these objects will then pick up the allergens. Proper precautions must be taken to ensure allergens are not spread.

b. **Housekeeping:** Decontaminate work surfaces before and after work and after spills. All animal areas should be kept neat and clean.

c. **Waste Disposal:** In some instances as required by the Institutional Biosafety Committee, bedding and other animal waste products will be collected by the Safety Department when contaminated with infectious agents. Animal Resources will incinerate noninfectious carcasses.

d. **Restraint of Animals:** Species specific safe techniques should be used to restrain animals. The use of mechanical restraint devices or chemical restraints can reduce the potential for escape or injury when animals are being examined or handled. Physical restraint can increase the inherent risks associated with the animal by intensifying excretions, secretions, and aggressive behavior of the animal. Animals injected with highly hazardous chemicals must either be restrained or anesthetized.

3. **Personal Protective Equipment (PPE)**

Personal protective equipment provides a physical barrier to materials that might otherwise come into contact with employees' skin, eyes, mucous membranes, and clothing. Selection should be based on specific knowledge of the potential hazard gained through training, experience and sound professional judgment. Laboratory coats and gloves should be worn as a minimum in protective clothing. Freshly laundered lab coats should be available daily. Gloves should be latex, nitrile, or rubber. NIOSH N95 respirators should be worn when exposure to aerosols may occur. When the animal study requires additional protection, such as the addition of a biological or chemical hazard, then the employee will be given specific instruction from the biosafety office on the type of additional protection needed.
B. Medical Evaluation and Preventive Medicine for Personnel

1. Medical Surveillance Questionnaires

All employees who handle animals must complete a Health Surveillance Questionnaire. Once an individual is identified on the IACUC protocol as a person who handles or has contact with live animals, the USC Safety Office will send an electronic message with information on how to complete the questionnaire. The questionnaire covers medical and occupational histories, to establish:

- Baseline health information identify pre-existing conditions that can affect the employee's capability to perform the essential functions of the position
- Report medical conditions that could alter the exposure risk profile, such as a female employee of childbearing age or susceptibility to allergies.
- The need for an examination and spirometry for employees wearing respirators.

Any work-related follow up examinations, immunizations, or treatments as a result of the medical surveillance questionnaire are covered by the USC occupational health program. Employees or their supervisors will not be expected to pay for any of these items.

2. Reporting of Illnesses

USC will not pay for the employee's primary care. However, if an employee is not certain as to the source of the illness an evaluation of the symptoms will be performed as a Workers Compensation case at no cost to the employee. Appropriate Workers Compensation forms must be filled out.

During the course of work, employees should report the following conditions when they occur:

- The development of allergies, asthma, seasonal rhinitis, or eczema.
- Any gastrointestinal, respiratory, or skin reactions.
- All bites, scratches, and other injuries to receive prompt medical attention.
- Any muscle or joint pain or discomfort which may be associated with handling cages or equipment should be reported.
- Any other symptoms that may indicate the onset of a possible work-related illness.

Contact the Biological Safety Specialist at 442-2200 for assistance.

3. Medical Management of Animal Bites, Scratches, or Other Adverse Health Outcomes

When an employee has received an animal bite or scratch, the employee must notify his or her supervisor immediately. The supervisor and the employee must complete incident reports for USC Worker's Compensation. The
supervisor will refer the employee to the USC Internal Medicine Department, AHC 100, 1355 San Pablo Street, 442-5100. For an evaluation on the University Park Campus, employees should go to the Student Health Center. After working hours and on weekends, occupational exposures will be seen at the Acute Care Center, 1500 San Pablo Street, in the Healthcare Consultation Center, at 2-5900.

Tetanus toxoid may be given and antibiotics may be warranted for significant bites. All medical evaluations will be performed and evaluated by a healthcare professional with an expertise in zoonoses.

4. Immunization, when appropriate

Tetanus toxoid is recommended at least every ten years. Bites from rodents are potentially a deep puncture wound for which tetanus would be a concern. All employees should evaluate their tetanus immunization status, and questions can be directed to the safety office.

5. Medical Evaluations For Staff Working with Nonhuman Primates

Research proposals for studies involving nonhuman primates must be approved by the Institutional Biosafety Committee. This committee will require additional medical evaluations for all staff that will handle nonhuman primates or have contact with nonhuman primate tissues. Evaluations include a physical examination, tuberculin testing, and other medical laboratory testing. Costs for these tests are covered as part of the USC occupational health program.
XII. References


2. Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996.


Appendix I

Signs of Pain and Distress

And

Recommended Analgesic Agents
## SIGNS OF PAIN OR DISTRESS

<table>
<thead>
<tr>
<th>Species</th>
<th>Mild to moderate pain / distress</th>
<th>Severe or chronic pain / distress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Eyelids partially closed; changes in respiration; rough hair coat; increased vibrissal movements; unusually apprehensive or aggressive; possible writhing, scratching, biting, self-mutilation; hunched posture; sudden running movements (escape); aggressive vocalization when handled or palpated; guarding</td>
<td>Weight loss; dehydration; incontinence; soiled hair coat; eyesunken, lids closed; wasting of muscles on back; sunken or distended abdomen; decreased vibrissal movements; unresponsive; separates from group; hunched posture; ataxia; circling; hypothermia; decreased vocalization</td>
</tr>
<tr>
<td>Rat</td>
<td>Eyelids partially closed; porphyrin staining around eyes; nose; rough hair coat +/- hair loss; increased aggression (toward humans and cage mates); reduced exploratory behavior; aggressive vocalization when handled; licking, biting, and/or scratching; guarding</td>
<td>Eyes closed; poor skin tone; muscle wasting along back; dehydration; weight loss; incontinence; soiled hair coat; depressed/unresponsive; sunken or distended abdomen; self-mutilation; recumbent position with head tucked into abdomen; decreased vocalization; hypothermia</td>
</tr>
<tr>
<td>Hamster</td>
<td>Ocular discharge; increased aggression (toward humans and cage mates); hunched posture; reluctance to move</td>
<td>Loss of coat and body condition; increasing depression; extended daytime sleep periods; lateral recumbency; hypothermia; sores on lips or paws</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Eyes sunken and dull; changes in respiration; increased timidity; increased sleepiness; arched back; increased vocalization when handled</td>
<td>Weight loss; hair loss; scaly skin; dehydration; decreased timidity; unresponsive; excessive salivation (oral problems); increased barbering; loss of righting reflex; decreased vocalization; hypothermia</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Ocular discharge; protruding nictitans; photophobia; constipation or diarrhea; depression; facing back of cage; excessive self-grooming; stretched posture; failure to eat and drink; dull attitude or increased aggression when handled; possible vocalization when handled; tooth grinding</td>
<td>Tooth grinding; apparent sleepiness; dehydration; weight loss; fecal staining; wasting of lower back muscles; decreased production of night feces; unresponsive</td>
</tr>
<tr>
<td>Nonhuman Primates</td>
<td>Generally very few signs, especially in the presence of humans; decreased activity; decreased food and water intake; agitation</td>
<td>Huddled or crouching posture, with hands folded over abdomen; clenching or grinding teeth; depression or increased restlessness; withdrawal from cage mates; increased (generally aggressive) attention from cage mates; anorexia; weight loss; decreased grooming</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dog</td>
<td>Decreased alertness; stiff posture; panting; biting; licking, or scratching; increased aggression; increased vocalization, especially when handled; decreased food &amp; water intake</td>
<td>Unwillingness to move; crouching posture; depression or increased aggression; crying when handled or moved; increased restlessness; loss of weight; decreased response to command</td>
</tr>
<tr>
<td>Pig</td>
<td>Changes in gait or posture; increased efforts to avoid handling; increased squealing when approached or handled</td>
<td>Depression; unwillingness to move; attempts to hide; withdrawal from pen mates; anorexia; change in body outline/profile; change in rooting mannerism</td>
</tr>
<tr>
<td>Bird</td>
<td>Increased escape behavior and vocalization when approached or handled</td>
<td>Eyelids partially closed; anorexia; ruffled, drooping, unkempt appearance; immobility when approached</td>
</tr>
</tbody>
</table>

**RECOMMENDED ANESTHESIA AND ANALGESIC AGENTS**

*These are general recommendations only and are not tailored regimens for any specific procedure, please seek DAR Veterinary guidance for modifications, other species or drugs than what is listed here.

**Mice and Rats**

**Anesthesia:**

<table>
<thead>
<tr>
<th>Mouse: Ketamine+Xylazine</th>
<th>80-100 mg/kg, 5-10 mg/kg</th>
<th>IP</th>
<th>Once</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: Ketamine + Xylazine</td>
<td>80-90 mg/kg, 5-10 mg/kg</td>
<td>IP</td>
<td>Once</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4%</td>
<td>inhalant</td>
<td>Continuous via nose cone</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4%</td>
<td>inhalant</td>
<td>Continuous via nose cone</td>
</tr>
<tr>
<td>Bupivicaine (0.5%)</td>
<td>1 ml/kg</td>
<td>Infiltration</td>
<td>For local anesthesia</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1-2 drops</td>
<td>Topical Ophthalmic</td>
<td>Once for ophthalmic use</td>
</tr>
</tbody>
</table>

**Analgesia:**

<p>| Buprenorphine | 0.02-0.05 mg/kg | SC | Every 6-12 hours for at least 48 hours post-op |</p>
<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage</th>
<th>Route</th>
<th>Frequency of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>1-2 mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1 drop</td>
<td>Topical Ophthalmic</td>
<td>Once</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5mg/kg</td>
<td>SC</td>
<td>Once a day</td>
</tr>
</tbody>
</table>

**Rabbit**

**Anesthesia:**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage</th>
<th>Route</th>
<th>Frequency of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine+Xylazine</td>
<td>35-50 mg/kg, 5-10 mg/kg</td>
<td>IM</td>
<td>Once</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>0.75-1.0 mg/kg</td>
<td>SC</td>
<td>Once</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4 %</td>
<td>Inhalant</td>
<td>Continuous via endotracheal intubation</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4 %</td>
<td>Inhalant</td>
<td>Continuous via endotracheal intubation</td>
</tr>
<tr>
<td>Bupivicaine (0.5%)</td>
<td>1 ml/kg</td>
<td>Infiltration</td>
<td>For local anesthesia</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1 -2 drops</td>
<td>Topical Ophthalmic</td>
<td>Once for ophthalmic use</td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage</th>
<th>Route</th>
<th>Frequency of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.01-0.05 mg/kg</td>
<td>SC</td>
<td>Every 12 hours for at least 48 hours post-op</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.1-0.3 mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
</tbody>
</table>

**Guinea Pig**

**Anesthesia:**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage</th>
<th>Route</th>
<th>Frequency of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine+Xylazine</td>
<td>20-40 mg/kg + 2 mg/kg</td>
<td>IM, IP</td>
<td>Once</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4%</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4 %</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
</tr>
<tr>
<td>Bupivicaine (0.5%)</td>
<td>1 ml/kg</td>
<td>Infiltration</td>
<td>For local anesthesia</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1 -2 drops</td>
<td>Topical Ophthalmic</td>
<td>Once for ophthalmic use</td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage</th>
<th>Route</th>
<th>Frequency of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.05 -0.1mg/kg</td>
<td>SC</td>
<td>Every 8-12 hours for at least 48 hours post-op</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.5-0.3mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
</tbody>
</table>
### Hamsters

**Anesthesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dose</th>
<th>Route</th>
<th>Method</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine+Xylazine</td>
<td>80mg/kg + 5 mg/kg</td>
<td>IM, IP</td>
<td>Continuous via nose cone</td>
<td>Once</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4%</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
<td></td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4%</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
<td></td>
</tr>
<tr>
<td>Bupivicaine (0.5%)</td>
<td>1 ml/kg</td>
<td>Infiltration</td>
<td>For local anesthesia</td>
<td></td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1-2 drops</td>
<td>Topical Ophthalmic</td>
<td>Once for ophthalmic use</td>
<td></td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dose</th>
<th>Route</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.05-0.5mg/kg</td>
<td>SC</td>
<td>Every 8 hours for at least 48 hours post-op</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.5-0. mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
</tbody>
</table>

### Gerbils

**Anesthesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dose</th>
<th>Route</th>
<th>Method</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine+Xylazine</td>
<td>50mg/kg + 2 mg/kg</td>
<td>IM, IP</td>
<td>Continuous via nose cone</td>
<td>Once</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4%</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
<td></td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4%</td>
<td>Inhalant</td>
<td>Continuous via nose cone</td>
<td></td>
</tr>
<tr>
<td>Bupivicaine (0.5%)</td>
<td>1 ml/kg</td>
<td>Infiltration</td>
<td>For local anesthesia</td>
<td></td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1-2 drops</td>
<td>Topical Ophthalmic</td>
<td>Once for ophthalmic use</td>
<td></td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dose</th>
<th>Route</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.1-0.2mg/kg</td>
<td>SC</td>
<td>Every 8 hours for at least 48 hours post-op</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.5-0. mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
</tbody>
</table>

### Non-Human Primates- *Macaca sp.*

**Anesthesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dose</th>
<th>Route</th>
<th>Method</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>5-20mg/kg</td>
<td>IM</td>
<td>Once</td>
<td></td>
</tr>
<tr>
<td>Ketamine/Medetomidine</td>
<td>3.0 mg/kg, 0.15 mg/kg</td>
<td>IM</td>
<td>Once</td>
<td></td>
</tr>
<tr>
<td>Ketamine/Diazepam</td>
<td>5-10mg/kg, 35mg/kg</td>
<td>IM</td>
<td>Once</td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-2% on 100% Oxygen</td>
<td>Inhalant</td>
<td>Continuous via endotracheal</td>
<td></td>
</tr>
</tbody>
</table>
**Intubation**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dosage</th>
<th>Route</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiopental</td>
<td>5-7 mg/kg</td>
<td>IV</td>
<td>To effect for induction</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>2-4% on 100% Oxygen</td>
<td>Inhalant</td>
<td>Continuous via endotracheal intubation</td>
</tr>
<tr>
<td>Propofol</td>
<td>2.5-5.0 mg/kg</td>
<td>IV</td>
<td>Bolus then infusion at 0.3-0.4 mg/kg/min</td>
</tr>
<tr>
<td>Telazol</td>
<td>1.5-3.0 mg/kg</td>
<td>IM</td>
<td>Induction</td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dosage</th>
<th>Route</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.01-0.02 mg/kg</td>
<td>IM, SQ</td>
<td>Every 12 hours for at least 48 hours post-op</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>7 mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.2 mg/kg</td>
<td>PO</td>
<td>Once a day</td>
</tr>
</tbody>
</table>

**Canine Anesthesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dosage</th>
<th>Route</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine</td>
<td>0.05-0.1 mg/kg</td>
<td>SC, IM, IV</td>
<td>Once</td>
</tr>
<tr>
<td>Propofol</td>
<td>2.5 – 5 mg/kg</td>
<td>IV</td>
<td>Bolus then infusion at 0.3-0.4 mg/kg/min</td>
</tr>
<tr>
<td>Thiopental</td>
<td>5 –20 mg/kg</td>
<td>IV</td>
<td>To effect</td>
</tr>
<tr>
<td>Ketamine /diazepam</td>
<td>5-10mg/.25-.5mg/kg</td>
<td>IV</td>
<td>To effect</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1-4%</td>
<td>Inhalation</td>
<td>Continuous via endotracheal intubation</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1-4%</td>
<td>Inhalation</td>
<td>Continuous via endotracheal intubation</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>(.5%) 1ml/kg</td>
<td>infiltration</td>
<td>For local anesthesia</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1-2 drops</td>
<td>ocular/ophthalmic</td>
<td>For ophthalmic use</td>
</tr>
</tbody>
</table>

**Analgesia:**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Dosage</th>
<th>Route</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>.01-.02 mg/kg</td>
<td>SC</td>
<td>Every 8-12 hrs</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2mg/kg</td>
<td>PO</td>
<td>Every 12 hrs</td>
</tr>
</tbody>
</table>

**Swine Anesthesia:**
Telazol/Xylazine 2.2 – 4.4 mg/kg IM For induction

Thiopental 5 – 20 mg/kg IV For induction

Isoflurane 1- 4 % inhalation Continuous via endotracheal intubation

Sevoflurane 1-4 % inhalation Continuous via endotracheal intubation

Bupivacaine (.5%) 1ml/kg infiltration For local anesthesia

Proparacaine 1-2 drops ophthalmic For ophthalmic use

** Analgesia:**

| Buprenorphine | 0.005 -0.02 mg/kg | IM | Every 8- 12 hrs |
| Carprofen | 2 – 4 mg/kg | PO | Every 12 hrs |

* PO - Oral
  IM- Intramuscular
  IV- Intravenous
  SQ- Subcutaneous
  IP- Intraperitoneal

** Frequency = time interval between doses.

References:
Anesthesia and Analgesia in Laboratory Animals
Laboratory Animal Medicine Second Edition
Formulary for Laboratory Animals Third Edition 2005