



**VA LONG BEACH HEALTHCARE SYSTEM
RESEARCH HEALTHCARE GROUP**

POLICIES AND PROCEDURES MANUAL

**FOR THE USE OF ANIMALS IN BIOMEDICAL
RESEARCH**

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SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

TRAINING OF RESEARCH PERSONNEL

The ANIMAL WELFARE ACT (federal law) mandates that:

"Training and instruction of research personnel must include guidance in at least the following areas:

- 1) Humane methods of animal maintenance and experimentation.
- 2) The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress.
- 3) Proper use of anesthetics, analgesics, and tranquilizers.
- 4) Methods whereby deficiencies in animal care and treatment are reported."

In addition the ILAR Guide for the Care and Use of Laboratory Animals (AAALAC accreditation standards) mandates that:

"Personnel caring for animals should be appropriately trained, and the institution should provide for formal or on-the-job training to facilitate effective implementation of the program and humane care and use of animals. Personnel using or caring for animals should also participate regularly in continuing education activities relevant to their responsibilities. On-the-job training should be part of every technician's job and should be supplemented with institution-sponsored discussion and training programs and with reference materials applicable to their jobs and the species with which they work. Investigators, technical personnel, trainees and visiting investigators who perform animal anesthesia, surgery, or other experimental manipulations must be qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner."

The Veterinary Medical Officer and VMU Staff are available for consultation and training at all times.

Questions concerning training should be directed to Steve Morris, VMU Animal Caretaker, extension 5822.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

REPORTING PERCEIVED DEFICIENCIES IN ANIMAL CARE AND TREATMENT

The Animal Welfare Act (federal law) requires that employees receive instruction on:

"methods whereby deficiencies in animal care and treatment are reported, including deficiencies in animal care and treatment reported by any employee of the facility. No facility employee, (SAS) Committee member or laboratory personnel shall be discriminated against or be subject to any reprisal for reporting violations of any regulation or standards under the act."

IT IS THE RESPONSIBILITY OF EACH AND EVERY PRIMARY INVESTIGATOR TO INSTRUCT ALL EMPLOYEES REPORTING TO THEM ON METHODS WHEREBY DEFICIENCIES IN ANIMAL CARE AND TREATMENT CAN BE REPORTED.

Any deficiencies in animal care and treatment at VMU may be reported (anonymously, if desired) to the following individuals:

Dr. Jeffrey L. Lee, Consulting Veterinarian Phone: (310) 222-3791 Fax: (310) 533-1158

Dr. Thay Lee, Chair, Subcommittee on Animal Studies Phone: (562) 494-5344.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

APPROVAL OF USE OF ANIMAL SUBJECTS

All animal studies, regardless of granting agency, formality of grant proposal, nature of study or any other general or specific feature, must be reviewed and approved by the SAS prior to the commencement of any activity related to the use of animals.

Process for SAS review of an "Animal Component of Research Protocol " form:

- 1) Consult with the Veterinary Medical Officer (VMO), Dr. Jeffrey L. Lee at (310) 222-3791.
- 2) The Principal Investigator (PI) completes an "Animal Component of Research Protocol" application form. Application forms are available through the VA Long Beach Healthcare System intranet site http://vaww.long-beach.med.va.gov/clinical_svc/research.htm. Assistance in completing the form may be obtained from Dr. Jeffrey L. Lee, or Dr. Thay Q. Lee.
- 3) The Principal Investigator electronically mails the completed application form to Dr. Jeffrey L. Lee at jlee@rei.edu. The Principal Investigator incorporates suggested changes, and forwards the ACORP and a copy of Dr. Lee's review comments to the Research and Development Office.
- 4) The finalized protocol is placed on the next Subcommittee on Animal Studies agenda. The application forms are distributed to all SAS members prior to the meeting.
- 5) The Principal Investigator is notified that the application form has been placed on the SAS meeting agenda. The Principal Investigator or a co-investigator named on the protocol is encouraged to be present at the SAS meeting.
- 6) At the SAS meeting, the SAS openly deliberates. Any additions, corrections or changes to the application form required by the SAS during its deliberations are noted in the record. A vote of SAS approval is always contingent on the Principal Investigator refiling any additions, corrections or changes of the application form with the SAS.
- 7) The Principal Investigator receives a signed, dated approved application form when Research & Development Committee approval is given to the protocol. If there are required application form changes at the SAS meeting, the Principal Investigator is notified in writing of the requested changes. Upon Principal Investigator resubmission of any required additions, corrections, or changes of the application form with the SAS, approval is given. If the application form is not approved or tabled, the Principal Investigator is notified in writing specifying the reason.
- 8) An application form may receive approval for three years. However, application forms for more than one year require re-approval on the anniversary date of the additional years. To achieve renewal, the Principal Investigator files with the SAS a "Subcommittee on Animal Studies Annual Progress Report." The renewal application form is reviewed by the SAS, following the same procedure for new application forms. All other aspects of corrections and voting are identical to that of the original application forms.

- 9) After the initial application has been approved by the SAS, modifications to the protocol may be requested by submitting a completed "Request for Modification/Addendum to Approved Project" form to the VMU Office. Forms may be obtained through the Research Office.

Questions pertaining to SAS application forms and submission deadlines should be referred to the Research Office at ext. 5801.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
ANNUAL REPORTS FOR USE OF ANIMAL SUBJECTS

The Subcommittee on Animal Studies grants approval for use of animal subjects for up to one year at a time, even when the Principal Investigator requests multi-year approval.

To renew the approval for use of animal subjects, the Principal Investigator must file a "Subcommittee on Animal Studies Annual Progress Report." application form by the submission deadline of the SAS meeting immediately prior to the expiration date of the approved application form. This short annual application form is in lieu of a complete filing, and will usually not require the Principal Investigator to attend an SAS meeting.

Approximately two months before the anniversary date of the R&D Committee approval for the original application, the Principal Investigator will receive the Annual Progress Report form. The Principal Investigator must file a completed annual application form or risk termination of animal use.

Any questions should be directed to the Research Office at ext. 5801.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

LATE PROGRESS REPORTS

A late progress report will result in a suspended protocol.

Access to animal subjects during suspension: The Subcommittee on Animal Studies administrative assistant will issue a notice to the Veterinary Officer, the VMU supervisor, and the PI informing them that pending reactivation of the protocol, access to the animal subjects is denied to the PI and his/her staff. Under no circumstances will experimentation occur during a suspended protocol. In cases when animals need treatment or if the animals must be euthanized, the VMU staff under the direction of the Consulting Veterinarian will perform all hands-on animal activities. Research administration will assess an appropriate fee for these activities

Reactivation of Protocol: Reactivation of the protocol will occur when the Subcommittee on Animal Studies has approved a progress report and any accompanying modification requests. The Subcommittee on Animal Studies administrative assistant will issue a notice to the Veterinary Officer, the VMU supervisor and the PI informing them that the protocol has been reactivated.

Per Diem: Per Diem will accrue at the full rate during the first month of suspension. On the 31st day of suspension, the Per Diem rate will increase to twice the standard rate.

Administrative Action after 60 days: Failure to submit a progress report within 60 days of the due date will result in administratively directed euthanasia of the animal subjects. When deemed appropriate by the Veterinary Officer and the Research Office, affected animal subjects may be offered to other AAALAC accredited institutions. The Subcommittee on Animal Studies will highly discourage reinstating protocols in projects with prior suspensions of 60 or more days.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
REQUEST FOR APPROVAL OF
MODIFICATION/ADDENDUM OF AN APPROVED PROTOCOL USING ANIMAL SUBJECTS

A "Subcommittee on Animal Studies Request for Modification/Addendum to Approved Project" (Protocol Modification) is to be filed by a Principal Investigator advising the SAS of changes in the care and use of live animals in an existing, approved protocol. Federal regulations governing NIH grants, and VA Long Beach Healthcare System policy governing all live animal research, require a procedure for institutional approval of changes in animal research (and the forwarding of institutional approval for NIH grants).

The definition of a change includes any and all alterations, no matter how great or small, in the care and use of live animals in research, testing or education. These include, but are not limited to, changes in personnel, animal numbers, anesthesia, surgical or non-surgical procedures or euthanasia. The boundary between what constitutes a change of an existing protocol and that which defines a new protocol may be ill-defined and variable. A change from the original direction of research involving animals, new Principal Investigator, or change of animal species, would usually require the filing of a complete and new "Animal Component of Research Protocol." Other changes, of a less substantive nature, would usually require only the filing of a protocol modification. If there is doubt about which form to file, the Principal Investigator is urged to consult with the Chairperson of the SAS or the consulting veterinarian (Dr. Jeffrey L. Lee).

In general, the material to be included with the filing should cover the same material required to answer those specific questions relating to the change as required in a new application. The Principal Investigator may choose to complete the sections of the original form "Animal Component of Research Protocol" which address the procedures being modified and attach this to the completed "Subcommittee on Animal Studies Request for Modification/ Addendum to Approved Project" form.

The consulting veterinarian and the Chairperson of the SAS or designee will promptly review the filing. In the majority of cases in which simple changes have been requested and the filing is complete, approval will be given within 48 hours, and the SAS will be notified at its next full meeting. Generally, a Principal Investigator need not be present at the SAS meeting for this review process. In unusual circumstances, the filing may be returned if the information supplied is incomplete or inappropriate; or, approval may be withheld pending full review by the SAS; or, the appearance of the Principal Investigator in front of the SAS may be required.

Completed protocol modification/addendum forms may be delivered to the Research and Development Office.

SPECIAL FEDERAL REQUIREMENTS RELATING TO
THE USE OF ANIMALS IN RESEARCH

Alternatives:

The Principle Investigator must consider alternatives to the proposed procedures in animals and must provide a written narrative description (in the SAS application) of the methods and sources used to determine that alternatives were not available.

Alternatives consist of the "3 R's" - Reduction, Refinement and Replacement.

Reduction in the number of animals used through sound study design to ensure the minimum number of animals necessary to achieve meaningful results is used.

Refinement of techniques to produce the least amount of stress in the animals.

Replacement of animals considered higher on the evolutionary scale with those considered lower on that scale or with non-living models (computers, tissue cultures, etc.)

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
QUARANTINE PROCEDURES FOR ANIMALS

- 1) Quarantine is the separation of newly received animals from those already in the facility until the health status of the newly received animals has been evaluated. Effective quarantine minimizes the introduction of disease agents into established colonies. In addition, the quarantine period permits the animals to adapt to their surroundings, resulting in a more stable physiological and behavioral state. The need for this stabilization period has been demonstrated in mice, rats, and guinea pigs; and is probably required for other species as well. Quarantine is necessary for all species of animals from all sources and though not used by the VMU, is especially predicated in random source animals (dogs, and rabbits). Random source animals are commonly infested with endoparasites (roundworms, hookworms, tapeworms, whipworms) and ectoparasites (fleas, ticks, mites) and may be afflicted with diseases such as upper respiratory infections, parvovirus, distemper, etc. In order to conduct sound biomedical research these disease entities must be identified and treated. Valid data cannot be collected from unhealthy animals.
- 2) By observing strict quarantine procedures, the Veterinary Staff will achieve the following:
 - diagnosis, control, prevention and treatment of diseases
 - physiological and nutritional stabilization
 - grooming, including bathing, dipping, and clipping as required
 - permanent identification (tattoo/ear tag): rabbits, dogs
- 3) To achieve these goals, incoming mice, rats, hamsters, and rabbits will be quarantined upon arrival for a minimum of three (3) working days (generally the day of arrival is not counted as a quarantine day). All dogs will be quarantined for a minimum of five (5) working days.
- 4) During the designated quarantine period, animals will not be available for experimentation.
- 5) The Veterinary Staff may release animals prior to the completion of quarantine for certain studies (i.e., euthanasia for tissue harvest). Request for early release of animals from quarantine must be made in advance to Dr. Jeffrey L. Lee (310) 222-3791.
- 6) The quarantine period for animals will be extended when necessary to diagnose, control, prevent or treat disease.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
ACCESSING RESEARCH ANIMALS ON WEEKENDS AND HOLIDAYS

The normal operational hours of the Veterinary Medical Unit are 8:00 a.m. to 4:30 p.m., Monday through Friday.

During weekends and holidays, one VMU employee is on-the-job from approximately 8:00 a.m. to 3:00 p.m.

- If researchers do access animals on weekends or holidays, under no circumstances are any unauthorized people (friends, family members, etc.) to enter any animal room.
- Under no circumstances will researchers be permitted to schedule weekend or holiday surgeries (survival or non-survival) on dogs, or rabbits.

In some cases, it may be acceptable for researchers to conduct rodent surgeries on weekends or holidays. However, in all cases prior arrangements must be made with the VMU Supervisor ext. 5822.

Under no circumstances are animals to be housed outside the VMU overnight, unless approved by Dr. Jeffrey L. Lee in advance.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
SURGERY IN NON-RODENT (RABBIT, DOG) MAMMALIAN SPECIES

A **Survival Surgery** is one from which the animal regains consciousness even if only momentarily.

A **Non-Survival Surgery** is one from which the animal never regains consciousness, as it is EUTHANATIZED while under anesthesia.

Aseptic technique must be used on all non-rodent mammalian species that undergo major or minor survival surgery. This technique includes wearing of sterile surgical gloves, gowns, caps and face masks; use of sterile instruments, and aseptic preparation of the surgical field (clipping of hair from incision site and application of a betadine solution).

Major survival surgery in non-rodent mammalian species must be performed in the Surgical Facility in the Research Building. **Major survival surgery** is defined as any surgical intervention that penetrates and exposes a body cavity (chest, abdomen, skull, joint, etc.) or that has the potential for producing a permanent handicap in an animal that is expected to recover.

Survival surgeries involving vascular cut-downs and catheterizations are examples of **minor survival surgeries**. These survival surgeries must be conducted using aseptic technique, however they can be performed in areas other than the surgical facility. **Non-survival surgeries** also may be performed in areas other than the surgical facility.

All surgical procedures must be performed or directly supervised by trained and experienced personnel in accordance with sound surgical practices. In all cases this involves:

Appropriate anesthesia (refer to "Anesthesia and Analgesic Guidelines for Research Animals," copy included in this manual). In survival surgical situations this involves, in addition to the above, the adherence to **Aseptic Technique Practices**.

Multiple survival surgical procedures on a single animal are discouraged, and **will not be permitted with out prior SAS approval**. SAS approval may be given if the multiple procedures are related components of a research protocol.

Post-surgical care must include observing the animal to ensure uneventful recovery from anesthesia and surgery accompanied by attention to wound care. The use of post-operative analgesics (refer to "Anesthesia and Analgesia Guidelines for Research Animals," copy included in this manual) is always indicated for major surgical procedures and may be indicated for minor surgical procedures. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved must be EUTHANATIZED at the end of the procedure, or if appropriate, during the procedure. Euthanasia must be performed by trained and experienced personnel, using acceptable techniques in accordance with the recommendations of the "AVMA Panel on Euthanasia" (copy available through VMU Office) as approved by the SAS.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
MULTIPLE SURGICAL USE OF ONE ANESTHETIZED ANIMAL

In some instances it may be appropriate and advantageous for two independent non-survival surgical procedures to be sequentially performed on a one-time anesthetized animal. Approval will be given for this type of usage only if the following criteria are met:

- 1) Only non-survival surgical procedures will be performed.
- 2) Only surgical procedures described in SAS approved research protocols will be performed.
- 3) All surgical procedures must be performed or directly supervised by trained and experienced personnel in accordance with sound surgical practices.
- 4) The multiple surgical use of a one-time anesthetized animal must be pre-scheduled. To accomplish this, please notify the VMU Office by **at least** 2:00 p.m. on the day prior to the proposed surgery. In addition, if the surgical procedures are to be performed in the VMU surgical suite, the use of the suite must be pre-scheduled through the VMU Office (ext. 5822).
- 4) If the first surgical procedure involves the use of radioactive, biohazardous, or carcinogenic materials, the primary investigators responsible for the first and second surgeries must consult with the Radiation Safety Officer (ext. 2880) and the Chair, Research Biohazard Committee (ext. 4079) for approval.
- 5) Researchers using radioactive, biohazardous, or carcinogenic materials during the conduct of the first or second surgical procedure will be responsible for appropriate disposal of the contaminated carcass.
- 6) Immediately following the first surgery, the surgeon responsible must complete an "Experimental Surgical Record" form and return this to the VMU Office. In addition, the surgeon responsible for the first surgery must complete the upper half of the "Notification of Multiple Use of One Anesthetized Animal" form (see attached copy) and turn this over to the surgeon performing the second procedure.
- 8) Immediately following the second surgery, the surgeon responsible must complete an "Experimental Surgical Record" plus the lower half of the "Notification of Multiple Use of One Anesthetized Animal" (see attached copy) and return both forms to the VMU Office.

PLEASE NOTE: If a one-time anesthetized animal is used for two independent protocols, the VMU Office will deduct one animal from the total number of animals approved for each protocol.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
SCHEDULING SURVIVAL SURGICAL PROCEDURES ON
NON-RODENT MAMMALIAN SPECIES

Because of difficulties in providing adequate veterinary and post-operative care to animals on weekends and holidays; scheduling survival surgical procedures in non-rodent mammalian species (dogs, and rabbits) on Fridays or other days prior to holidays is discouraged.

If survival surgical procedures must be conducted on Fridays or days prior to holidays (and veterinary approval is given), the surgery must be completed and the patient returned to the VMU no later than 3:30 p.m. Failure to comply with this policy will result in denial of future requests to perform survival surgery in non-rodent mammals on Fridays or other days prior to holidays.

Immediately after surgery, an "Experimental Surgical Record" form must be completed and returned to the Vivarium with the animal.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

RODENT SURGERY

A Survival Surgery is one from which the animal regains consciousness, even if only momentarily.

A Non-Survival Surgery is one from which the animal never regains consciousness as it is EUTHANATIZED while deeply anesthetized.

A Rodent Surgical Area (used for either survival or non-survival surgery) may be a room or portion of a room that is easily sanitized; it must be maintained and operated to ensure cleanliness. It should not be used for any other purpose.

All surgical procedures must be performed or directly supervised by trained and experienced personnel in accordance with sound surgical practices. In all cases this involves:

Appropriate anesthesia (refer to "Anesthesia and Analgesia Guidelines for Research Animals," copy included in this manual)

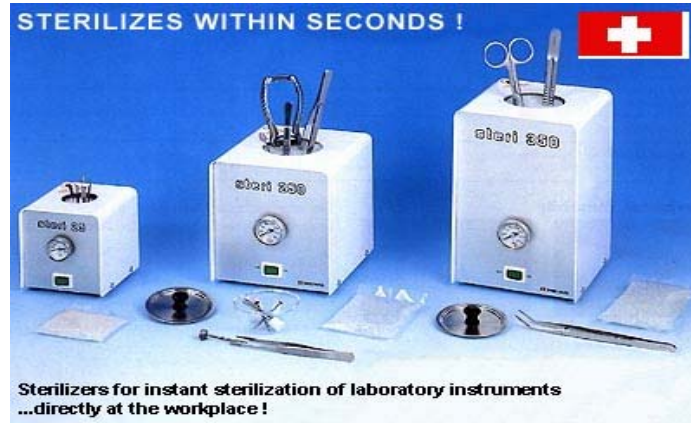
Specifically in Survival Surgical situations this involves (in addition to the above):

- **Appropriate preparation of the surgical field** (removal of hair and application of a betadine prep.)
- **Use of sterile instruments** (cold sterilization is acceptable); Note: Alcohol is not a sterilizing medium.
- **Wearing of sterile surgical gloves.**
- **Use of aseptic technique.**

Multiple survival surgical procedures on a single animal are discouraged, and **will not be permitted without prior SAS approval**. SAS approval may be given if the multiple procedures are related components of a research protocol.

Post-surgical care must include observing the animal to ensure uneventful recovery from anesthesia and surgery accompanied by attention to wound care. The use of analgesics post-operatively (refer to "Anesthesia and Analgesia Guidelines for Research Animals," copy included in this manual) is always indicated for major surgical procedures and may be indicated for minor surgical procedures. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved must be EUTHANATIZED at the end of the procedure, or if appropriate during the procedure. Euthanasia must be performed by trained and experienced personnel; using acceptable techniques in accordance with the recommendations of the "AVMA Panel on Euthanasia" (copy available through VMU Office) as approved by the SAS.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
USE OF HOT-BEAD STERILIZER



Two Hot-Sterilizers are available at the VMU office, B-38, for all researchers.

INSTRUCTIONS

1. Please check the container to make sure that the glass beads are filled 2 mm shorter than its rim. Make sure that the beads are dry and clean. Fill more beads if necessary. Additional beads are available at VMU Office. Never fill the container with other material or liquids.
2. Switch on the sterilizer. After the heating-up time, the inner temperature reaches 250°C (482°F), which is maintained as long as the sterilizer is switched on (+/- 5%). **DO NOT TOUCH THE GLASSBEADS OR GLASSBEAD CONTAINER BEFORE COOLING DOWN!**
3. Remove the metal cover. To allow good ventilation of the outer case, do not place the metal cover back on the glass bead container until the sterilizer is switched off.
4. Insert dry and clean instruments into the glass beads as deep as possible for at least 10 seconds. Remove instruments carefully because they are very hot.
5. The sterilizer can remain in constant use all day with no overheating.
6. After use, switch off the sterilizer. Place the metal cover on the glass bead container to keep dust and foreign bodies away and return it to the VMU office.
7. Call the VMU office at 5822 for questions or sterilizer availability.

RECALL THAT ALCOHOL IS NEITHER A STERILANT NOR A DISINFECTANT.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

ANIMAL EUTHANASIA

Euthanasia is the act of inducing painless death. Criteria to be considered for a painless death are: rapid occurring unconsciousness and unconsciousness followed by cardiac or respiratory arrest.

Techniques for euthanasia should follow current guidelines established by the American Veterinary Medical Association (AVMA) Panel on Euthanasia. Copies of the AVMA Panel on Euthanasia are available through VMU Office (ext. 5822). Any deviation from the AVMA Panel on Euthanasia must receive approval from the VA Long Beach Healthcare System SAS.

The most common means of euthanasia for species of animals used at VA Long Beach Healthcare System include:

Rodents (Mice, Rats, Hamsters, Guinea Pigs)

CO₂ inhalation chamber (gas cylinder preferred over dry ice)

Note: CO₂ is not effective euthanasia for neonatal rodents! CO₂ should be delivered slowly to the chamber to avoid excitation and stress in the rodent to be EUTHANATIZED.

Anesthetic overdose (IV or IP)

Tissue harvest resulting in exsanguination under deep surgical anesthesia

Euthanasia Solution (Commercial Veterinary Product) IV or IP

Note: Cervical dislocation and/or decapitation without prior anesthesia or sedation will not be approved by the SAS without scientific justification supported by literature references and review of technique by veterinary staff.

Rabbits, and Dogs

IV anesthetic overdose

IV KCl during deep surgical anesthesia

IV Commercial Veterinary Euthanasia Solution

Tissue harvest resulting in exsanguination under deep surgical anesthesia

Questions concerning euthanasia procedures and techniques should be referred to Dr. Jeffrey L. Lee, Veterinary Medical Officer (310) 222-3791.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
TISSUE HARVESTING FROM EUTHANATIZED ANIMALS

The Subcommittee on Animal Studies encourages tissue harvesting from EUTHANATIZED animals as a method of reducing the total number of animals used in approved biomedical research. The VMU Office will keep on file an up-to-date list of projects, animal species and protocol information to assist investigators in identifying potential sources of tissue for harvesting.

All aspects of rules and regulations for the care and use of animals will be maintained. Investigators with approved protocols are permitted to harvest tissues from animals at the time of euthanasia. Other investigators not listed on the approved protocol may also harvest tissue from the EUTHANATIZED animal with the approval of the Principal Investigator of the protocol, who retains primary authority and responsibility for the care and use of animals. However, the following stipulations must be observed:

- 1) If any aspect of the care and use of the live animal before euthanasia, or any aspect of its euthanasia/carcass disposal is modified to meet the requirements of tissue harvesting, the original Principal Investigator of the approved protocol must file a "Request for Modification" form and receive approval of the SAS. The form shall include the modification, its purpose and the name(s) of the other investigator(s) who intend to harvest tissue.
- 2) If the carcass contains radioactive, hazardous, infectious or recombinant DNA material, other investigators who harvest tissue from the carcass must file a "Radiation Safety or Biohazard" form and receive approval from the Radiation and Environmental Safety Office.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON
THE USE OF COMPLETE FREUND'S ADJUVANT (CFA)

CFA, an oil-in-water emulsion containing mycobacterial cell wall components, is regarded scientifically as an effective means of potentiating humoral antibody response to injected immunogens. Adjuvant activity is a result of sustained release of antigen from the oily deposit and stimulation of a local immune response. An essential component of this response is an inflammatory reaction at the site of antigen deposition resulting from the sequestration of immunoreactive cells and their interaction with antigen.

The use of CFA is, therefore, an important biologic resource for investigators which should be used responsibly and with care to avoid or minimize the adverse effects of excessive inflammation. Undesirable and painful side effects such as large inflammatory lesions or tissue necrosis can be effectively reduced or eliminated by the use of appropriate routes of administration, adequate separation of injection sites, and the use of a small amount of inoculum per site.

The following are guidelines for the use of CFA in research animals at VA Long Beach Healthcare System:

- 1) Prior to injection of CFA, the injection site must be clipped free of hair and prepped with betadine.
- 2) CFA is to be injected subcutaneously (never intradermally, intramuscularly or intraperitoneally).
- 3) In rabbits, no more than 0.1 cc to 0.3 cc of CFA is to be injected at any given site.
- 4) In rodents, only up to 0.1 cc of CFA can be injected at any given site.
- 5) CFA may be used in any given animal only once. Booster injections, if required, must contain only Incomplete Freund's Adjuvant (an oil-in-water emulsion lacking mycobacterial components).
- 6) Use of CFA must be approved by the SAS.
- 7) Any proposed deviation from this policy must be adequately justified and approved by the SAS.

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

BLOOD WITHDRAWAL VOLUMES AND FREQUENCY OF BLOOD COLLECTION

As a general rule, approximately 7.0 % of an animal's body weight is comprised of blood.

Examples:

- 1) 55 lb (25 kg) dog
7.0 % of 25 kg = 1.75 kg = 1750 ml
- 2) 10 lb (4.54 kg) rabbit
7.0 % of 4.54 kg = 0.31 kg = 310 ml
- 3) 40 g mouse
7.0 % of 40 g = 2.8 g = 2.8 ml

As a general rule, 15 % of an animal's total blood volume may be safely removed without severely harming the animal (of course, fluid replacement therapy must be instituted if this maximum volume is removed.)

Examples:

- 1) 55 lb (25 kg) dog: 7 % of body weight = 1750 ml blood
15 % of 1750 ml = 262 ml
- 2) 10 lb (4.54 kg) rabbit: 7 % of body weight = 310 ml
15 % of 310 ml = 48 ml
- 3) 40 g mouse: 7 % of body weight = 2.8 ml
15 % of 2.8 ml = 0.42 ml

As a general rule, animals will replace lost blood at a rate of 1ml per pound body weight per 24 hours (1ml/lb / 24 hr).

Examples:

- 1) 55 lb dog will produce 55 ml of blood in 24 hours, 110 ml of blood in 48 hours, etc.
- 2) 10 lb rabbit will produce 10 ml of blood in 24 hours, 20 ml of blood in 48 hours, etc.
- 3) 40 g mouse will produce 0.08 ml of blood in 24 hours, 0.16 ml of blood in 48 hours, etc.

Animals cannot be bled at a volume and frequency which does not allow them to replace their blood volume.

Example: 10 lb (4.54 kg) rabbit: if 50 ml of blood is drawn (maximum safe bleed), this amount cannot be taken again for another 5 to 7 days (1 ml/lb / 24 hr at 10 lb = 5 days).

Direct questions concerning blood withdrawal from animals to Dr. Jeffrey L. Lee.

INNOVAR RECOMMENDED USE

(For use in rabbits for vasodilation of central auricular artery)

- 1) Inject Innovar subcutaneously at a dose of 0.125 ml/kg body weight or 0.5ml for an average 4kg rabbit.
- 2) Let animal remain in cage until vasodilation occurs, about 20 minutes.
- 3) Place rabbit in a restrainer cage and clip or shave the dorsal aspect of the ear over the artery.
- 4) Collect blood sample as needed.
- 5) When sample has been collected, remove needle and apply direct pressure over the artery.

It is important that Innovar be injected subcutaneously; the intramuscular route may cause lameness and local necrosis.

ANESTHETIC AND ANALGESIC GUIDELINES FOR RESEARCH ANIMALS

ABBREVIATIONS

hr	hour
IM	intramuscularly
IP	intraperitoneally
IV	intravenously
Kg	kilogram
mcg	microgram
mg	milligram
PO	per os (orally)
q	every
SC	subcutaneously

SUBCOMMITTEE ON ANIMAL STUDIES (SAS) POLICY ON

**USE OF ANESTHETICS AND ANALGESICS IN
SURVIVAL AND NON-SURVIVAL SURGERIES**

Expired anesthetics and analgesics are not authorized for use in either survival or non-survival surgeries. Laboratory staff should be aware of the expiration date on all. Dispose of expired anesthetics and analgesics by contacting the Supervisor in the Veterinary Medical Unit.

Note the expiration date from the original vial on all secondary containers used for administration or dilution.

ANESTHETIC AND ANALGESIC GUIDELINES FOR DOGS

Analgesics

Aspirin	10.5 - 25.0 mg/Kg PO q12hr
Buprenorphine*	0.01 - 0.02 mg/Kg IM, SC q12hr
Butorphanol	1.0 - 2.0 mg/Kg SC, IM, IV
Meperidine	0.5 - 1.0 mg/Kg IM, IV q1hr
Morphine	0.5 - 5.0 mg/Kg IM, SC q4hr
Pentazocine	2.0 mg/Kg IM, SC q5hr
Phenylbutazone	22.0 mg/Kg IV q8hr or 10 mg/Kg PO q8hr

Anesthetics (Injectable)

Ketamine and Xylazine	6.0 - 10.0 mg/Kg and 2.0 - 3.0 mg/Kg IM
Comment:	Should be accompanied by Atropine 0.05 mg/Kg IM, SC to decrease respiratory secretions
Ketamine and Medetomidine	2.5 - 7.5 mg/Kg and 80 mcg/Kg IM
(reverse Medetomidine with Atipamezole)	1.0 mg/Kg IV/SC)
Pentobarbital	20.0 - 30.0 mg/Kg IV
Comment:	Not recommended for survival surgeries; respiratory depression requires endotracheal intubation and ventilation
Thiopental*	10.0 - 30.0 mg/Kg IV
Comment:	Ultrashort (15 minute) anesthetic, used primarily as anesthesia induction agent prior to inhalational anesthesia
Telazol (Tiletamine/Zolazepam)	6.0 - 13.0 mg/Kg IM

Anesthetics (Inhalation)

Halothane	Comment: Requires anesthesia machine
Methoxyflurane	Comment: Requires anesthesia machine
Isoflurane*	Comment: Requires anesthesia machine
Nitrous Oxide	Comment: Must be used in combination with other anesthetic
Enflurane, Fluothane, Isoflurane	Comment: Requires anesthesia machine

*Most commonly used

ANESTHETIC AND ANALGESIC GUIDELINES FOR DOGS (continued)

Immobilizing Agents

Ketamine 6.0 - 10.0 mg/Kg IM

Sedatives/Tranquilizers

Acepromazine 0.5 - 1.0 mg/Kg IM, SC

Chlorpromazine 3.0 - 5.0 mg/Kg IM

Diazepam 5.0 - 10.0 mg/Kg IM

Medetomidine 10 - 80 mcg/Kg SC/IM/IV

(reverse with atipamezole 1mcg/Kg SC/IV)

Telazol (Teletamine/Zolazepam) 7.0 -9.0 mg/Kg IM

Miscellaneous

Bretylium tosylate 3.0 - 5.0 mg/Kg IM, IV

Furosemide (Lasix) 2.0 - 4.0 mg IV, IM, PO SID-TID

Heparin 200 u/Kg IV, SC

Lidocaine 1.0 - 2.0 mg/Kg IV

Procainamide 6.0 - 20.0 mg/Kg IM, SID-QID

Digoxin (maintenance) 0.01 - 0.02 mg/Kg PO divided BID

(loading) 0.02 - 0.06 mg/Kg PO divided BID

Yohimbine (reverses Xylazine) 0.1 mg/Kg IV

ANESTHETIC AND ANALGESIC GUIDELINES FOR MICE

Analgesics

Buprenorphine*	0.05 - 0.1 mg/Kg SC q12hr
Butorphanol	5.4 mg/Kg SC
Meperidine	20.0 mg/Kg SC q1hr
Morphine	10.0 mg/Kg SC
Pentazocine	10.0 mg/Kg SC, IV q5hr
Phenylbutazone	200.0 mg/Kg PO

Anesthetics (Injectable)

Ketamine and Xylazine and Acepromazine	30.0 mg/Kg IP and 6.0 mg/Kg IP and 1.0 mg/Kg IP
Ketamine and Xylazine*	40.0 - 50.0 mg/Kg and 40.0 - 50.0 mg/Kg IP
Ketamine and Medetomidine	75.0 mg/Kg and 1.0 mg/Kg IP
(reverse Medetomidine with Atipamezole	1.0 mg/Kg SC/IP/IV)
Pentobarbital	30.0 - 90.0 mg/Kg IP 25.0 - 50.0 mg/Kg IV
(Comment: High mortality rate at high doses; not recommended)	
Pentobarbital/Ethanol Cocktail:	
70.0% Ethanol	1.0 cc
Sterile (distilled) Water	6.0 cc
65.0 mg/ml Pentobarbital	<u>0.8 cc</u>
Administer	0.01 cc/gm IP
Thiopental	25.0 - 50.0 mg/Kg IV
Tribromoethanol/amylene hydrate (Avertin)	0.2 ml/10g BW IP of 1.25% solution

Anesthetics (Inhalation)

Methoxyflurane

Comment: Requires bell jar, nose cone, and effective scavenging of waste gasses (fume hood). Provision must be made to prevent direct contact of animal with liquid methoxyflurane.

Immobilizing Agents

Ketamine	100.0 - 200.0 mg/Kg SC 25.0 - 50.0 mg/Kg IP
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*Most commonly used

ANESTHETIC AND ANALGESIC GUIDELINES FOR MICE (continued)

Ketamine and
Acepromazine 100.0 mg/Kg and
2.5 mg/Kg SC

Sedatives/Tranquilizers

Acepromazine 15.0 mg/Kg SC
5.0 mg/Kg IP
Chlorpromazine 6.0 - 12.0 mg/Kg IP
25.0 mg/Kg SC
Diazepam 5.0 - 6.0 mg/Kg IP
Innovar-Vet 1:9 dilution with saline .00125 cc/gm SC
Xylazine 10.0 - 15.0 mg/Kg SC
Medetomidine 30.0 - 100.0 mcg/Kg SC
(reverse with Atipamezole 1 mcg/Kg SC/IP)

Miscellaneous

Pregnant Mares Serum (PMS) 51 u/mouse IP
Human Chorionic Gonadotropin (HCG) 51 u/mouse IP

Recommendation for Injectable Anesthesia:

Ketamine 50 mg/Kg and Xylazine 50 mg/Kg IP

Need: Ketamine 10 mg/ml
0.4 cc Ketamine (100 mg/ml)
3.6 cc H₂O
4.0 cc Ketamine 10 mg/ml

Xylazine 10 mg/ml
2.0 cc Xylazine (20 mg/ml)
2.1 cc H₂O
4.0 cc Xylazine 10 mg/ml

Administer 0.05 cc/10g BW of each Ketamine and Xylazine IP

Example: 30g mouse

0.15 cc Ketamine
0.16 cc Xylazine
0.17 cc Combination IP

ANESTHETIC AND ANALGESIC GUIDELINES FOR RABBITS

Analgesics

Buprenorphine*	0.02 - 0.05 mg/Kg SC q12hr
Meperidine	5.0 - 10.0 mg/Kg IM, SC
Morphine	5.0 - 10.0 mg/Kg IM, IP, SC q4hr
	2.0 - 5.0 mg/Kg IV
Pentazocine	10.0 20.0 mg/Kg SC q4-6hr
Phenylbutazone	100.0 mg/Kg IV

Anesthesia (Injectable)

Ketamine and Xylazine	10.0 mg/Kg and 3.0 mg/Kg IV
Comment:	IV anesthetics should be diluted 3:1 with saline and administered very slowly to effect
Ketamine and Xylazine	35.0 - 50.0 mg/Kg and 5.0 - 8.0 mg/Kg SC
Ketamine and Xylazine and Acepromazine	35.0 mg/Kg and 6.0 mg/Kg and 0.75 mg/Kg SC
Comment:	Should be accompanied by Atropine 0.2 mg/Kg SC to decrease respiratory secretions
Ketamine and Medetomidine	25.0 mg/Kg and 0.5 mg/Kg IM/SC
	(reverse Medetomidine with Atipamezole 1.0 mg/Kg SC/IV)
Ketamine and Medetomidine	5.0 mg/Kg and 0.35 mg/Kg IV
	(reverse Medetomidine with Atipamezole 1.0 mg/Kg SC/IV)
Pentobarbital	15.0 - 40.0 mg/Kg IV
	40.0 mg/Kg IP
Comment:	High mortality at higher doses - not recommended
Thiamylal	15.0 - 25.0 mg/Kg IV
Comment:	Ultrashort (15 minute) anesthetic
Thiopental	20.0 - 50.0 mg/Kg IV
Comment:	Ultrashort (15 minute) anesthetic

Anesthetics (Inhalation)

Halothane	
Comment:	Requires precision vaporizer

*Most commonly used

ANESTHETIC AND ANALGESIC GUIDELINES FOR RABBITS (continued)

Isoflurane

Comment: Requires anesthesia machine

Methoxyflurane

Comment: Can maintain with nose cone only after anesthesia is induced with another agent

Immobilizing Agents

Ketamine 30.0 - 75.0 mg/Kg SC

Ketamine and 30.0 - 70.0 mg/Kg and

Acepromazine 2.0 - 5.0 mg/Kg SC

Comment: Should be accompanied by Atropine 0.2 mg/Kg SC to decrease respiratory secretions

Sedatives/Tranquilizers

Acepromazine 1.0 mg/Kg SC

Diazepam 4.0 - 5.0 mg/Kg SC

Xylazine 3.0 - 6.0 mg/Kg SC

Medetomidine 100.0 - 500.0 mcg/Kg SC

(reverse with Atipamezole 1 mcg/Kg SC/IV)

Vasodilator

Innovar-Vet .125 ml/Kg SC

(See Recommendation for Use of Innovar-Vet in this manual)

Miscellaneous

Heparin 200 u/Kg IV, SC

Yohimbine (reverse Xylazine) 0.2 mg/Kg IV

Surgical Fluid Replacement

Administer 10 ml/lb body weight/hr IV

ANESTHETIC AND ANALGESIC GUIDELINES FOR RATS

Analgesics

Buprenorphine*	0.05 - 1.0 mg/Kg SC q12hr
Butorphanol	23.0 mg/Kg SC
Morphine	5.0 - 10.0 mg/Kg SC q4hr
Meperidine	20.0 mg/Kg SC q1hr
Pentazocine	5.0 - 10.0 mg/Kg SC, IV q1-4hr
Phenylbutazone	200.0 mg/Kg PO

Anesthetics (Injectable)

Ketamine and Xylazine*	45.0 - 100.0 mg/Kg and 5.0 - 10.0 mg/Kg IP, SC
Comment:	Should be accompanied by Atropine 0.05 mg/Kg SC, IP to decrease respiratory secretions.
Ketamine and Medetomidine	75.0 mg/Kg and 0.5 mg/Kg IP
(reverse Medetomidine with Atipamezole	1.0 mg/Kg SC/IP/IV)
Inactin	80.0 - 110.0 mg/Kg IP
Pentobarbital	25.0 - 40.0 mg/Kg IP, IV
Comment:	High mortality at higher doses - not recommended.
Telazol (Telatamine/Zolazepam)	20.0 - 40.0 mg/Kg IP
Thiopental	20.0 - 40.0 mg/Kg IP
	30.0 mg/Kg IV
Comment:	Short (15 minute) duration
Tribromoethanol/Amylene Hydrate	300 mg/Kg IP

Anesthetics (Inhalation)

Halothane	
Comment:	Requires precision vaporizer
Methoxyflurane*	
Comment:	Requires bell jar, nose cone, and effective scavenging of waste gasses (fume hood). Provision must be made to prevent direct contact of animal with liquid methoxyflurane.

Immobilizing Agents

Ketamine	60.0 - 100.0 mg/Kg SC, IP
Ketamine and Acepromazine	45.0 - 75.0 mg/Kg and 2.5 - 5.5 mg/Kg SC
Comment:	Should be accompanied by Atropine 0.05 mg/Kg SC, IP to decrease respiratory secretions.

*Most commonly used

ANESTHETIC AND ANALGESIC GUIDELINES FOR RATS (continued)

Sedatives/Tranquilizers

Acepromazine	5.0 mg/Kg SC
Chlorpromazine	5.0 - 20.0 mg/Kg IP
Diazepam	2.5 - 10.0 mg/Kg IP
Innovar-Vet	.1 cc/Kg SC
Ketamine	20.0 mg/Kg IP
Xylazine	15.0 mg/Kg SC
Medetomidine	30.0 - 100.0 mcg/Kg SC
(reverse with Atipamezole 1.0 mcg/Kg SC/IP/IV)	

Miscellaneous

Heparin	200 u/Kg SC
Curari	.075 mg/Kg IV
Yohimbine (reverse Xylazine)	1.0 - 2.0 mg/Kg SC/IP

Recommendation for Injectable Anesthesia

Ketamine 100 mg/Kg and Xylazine 10 mg/Kg and Atropine .05 mg/Kg

Need: Ketamine 100 mg/ml
Xylazine 20 mg/ml
Atropine .054 mg/ml

0.4 cc Atropine (.54 mg/ml)
3.6 cc H₂O
4.0 cc Atropine (.054 mg/ml)

Administer: Ketamine 0.1 cc/100g BW
Xylazine 0.05 cc/100g BW
Atropine 0.1 cc/100g BW

Example: 200g rat

0.2cc Ketamine
0.1cc Xylazine
0.2cc Atropine
0.5cc Combination IP

Option: Administer $\frac{3}{4}$ of dose and observe for five minutes.