University Committee on Animal Resources

Manual on the Responsible Care and Use of Laboratory Animals

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Preface

The cornerstone of responsible care and use of laboratory animals in a research facility is an institutional commitment to a strong training and continuing education program. The dynamic nature of biomedical research requires that we keep abreast of changes in regulations and refinements in research techniques. The University of Rochester Manual On The Responsible Care And Use of Laboratory Animals guides researchers through existing regulations and instructs personnel about humane methods of animal maintenance and experimentation. The Manual is one part of a multifaceted training program available to research personnel and animal care technicians at the University of Rochester. Individual training in specific techniques of biomethodology is available for researchers contemplating a new animal model or developing an experimental technique. A periodic Newsletter and updated pages to the Manual will keep you informed of legislative trends, aware of animal care and use issues at the University of Rochester, and current with new techniques in laboratory animal research. I look forward to visiting your animal laboratories and becoming more familiar with your areas of research. Our goal is to contribute to an environment where the highest quality biomedical research and teaching are possible.

Sincerely,

Jeff Wyatt D.V.M., M.P.H. ACLAM Diplomate
Director and Chair of Comparative Medicine, University of Rochester
University of Rochester Attending Veterinarian

Chapter 1: Regulations & Requirements

Introduction

Scientists working with laboratory animals in research or teaching protocols may find the long list of acronyms used to describe regulatory, institutional and funding agencies overwhelming. The Institutional Animal Care and Use Committee (IACUC), Public Health Service (PHS), United States Department of Agriculture (USDA), and New York State Department of Health (NYSDH) play instrumental roles in laboratory animal care and use at the University of Rochester. The purpose of Chapter 1 is to introduce the organizations that promulgate regulations and requirements we must be familiar with to maintain the license and privilege to use laboratory animals in biomedical research.

Institutional Animal Care and Use Committee (IACUC)

The Institutional Animal Care and Use Committee (IACUC) is required by federal law and as a condition of NIH funding. The University of Rochester IACUC is the University Committee on Animal Resources (UCAR). UCAR must have at least five members, including a veterinarian with program responsibilities, a scientist experienced in laboratory animal research, a non-scientist and an individual who has no affiliation with the institution besides UCAR membership. The following UCAR functions are mandated by the USDA through the Animal Welfare Act regulations or required by the Public Health Service as a condition of NIH funding.

1. UCAR reviews proposed research protocols for compliance with USDA Regulations and PHS Policy and guidelines. The principal investigator submits the "Authorization to Use Animals in Research or Testing" form to UCAR (please refer to the forms at http://www.urmc.rochester.edu/ucar/forms/index.cfm and the guidelines for forms submission at http://www.urmc.rochester.edu/forms/guidelines.cfm). UCAR may approve or require more information or modifications before approval. All animal research and testing protocols must be prospectively reviewed and approved by UCAR.
2. UCAR reviews all approved protocols annually. The UCAR secretary sends the UCAR Annual Protocol Review to the principal investigator for completion before the anniversary date of the approval. All protocols must be reviewed and approved annually. Full protocol review occurs every three years. Any protocol modification must be prospectively approved.
3. UCAR must inspect, at least once every six months, all of the animal facilities, including animal study areas/satellite facilities, using the USDA Regulations and PHS Guide as basis. UCAR submits a written report of the inspection to the Institutional Official. The Acting Institutional Official (Dr. Mark Taubman) is the individual at a research facility who is authorized to legally commit on behalf of the research facility that the requirements of the federal regulations are met. UCAR suspends an activity involving animals when necessary; takes corrective action and reports to the funding agency and/or USDA.
4. UCAR makes recommendations to the Institutional Official regarding any aspect of the research facility's animal program, facilities or personnel training.

Please contact the UCAR secretary at x5-1693 to schedule a meeting for assistance or more information.

Authorization to Use Animals in Research or Teaching

Federal and State statues as well as the Public Health Service Animal Welfare Policy require that the University establish an "Institutional Animal Care and Use Committee," hereafter known as the University Committee on Animal Resources (UCAR). Under regulatory mandates UCAR is charged with the responsibility and authority for oversight of proper care and use of all laboratory animals. As part of meeting this responsibility, the University requires that all laboratory animal use be reviewed and approved by UCAR.

In order to insure that all projects requiring the use of laboratory animals have been adequately reviewed, according to the specific criteria detailed in the federal regulations, UCAR has adopted the "Animal Use Protocol" FORM. A FORM must be completed, reviewed and approved prior to placing an order for animals.

The FORM must be submitted and signed by a Faculty Member of the University of Rochester as the Principal Investigator (P.I.). Non-faculty members, e.g. post-doctoral fellows, graduate students, etc. may be named as Co-PI.

The primary purpose of the UCAR review is to be sure that animal welfare concerns have been adequately considered. This usually occurs prior to review of the scientific merit of the grant application by external funding agencies. The Public Health Service (PHS), including NIH, allows for up to 60-days after grant deadline for this approval to be given.

Care should be taken when filling out this FORM to assure completeness and accuracy. The FORM must be typed. Please refer to the Guidelines within the form and the "Extended Help" to assist in completion of this form. UCAR recognizes that research is creative and dynamic, however, during the course of a project, significant variances must be justified and described in writing to UCAR and prospectively approved by UCAR.

According to federal mandate, approvals may only be given for one year. For annual renewals, a short questionnaire must be completed and filed with the UCAR if no significant change in the protocol or personnel has occurred. A new application, review and approval by UCAR is required every three years whether or not significant changes have been made.

Please answer all questions on the FORM. Do not attach a grant application or reference it. Do not reference a previously approved protocol or part of a protocol or procedure that has been previously approved. Do not refer to other pages in the FORM or grant proposal itself.

For assistance in completing the FORM, please contact the UCAR Executive Secretary, x5-1693 between the hours of 7:30 AM and 4:00 PM.

Guidelines to Use During Completion of Animal Care and Use Forms

WEB-Based Submission of Animal Protocols – Animal Use Protocols must be submitted online using Topaz Enterprise – beginning January 1, 2010, all new submissions of animal protocols and all 36-month reviews must be submitted in this format. UCAR has been accepting submissions on this system for about six months. The Topaz System can be accessed by web browsers (Internet Explorer, Firefox, Safari) from either a Mac or PC.

TOPAZ training is mandatory – all faculty and staff must attend a TOPAZ training session and obtain an account. New faculty members who are transferring to the University must also submit protocols online (individual training can be arranged via telephone if necessary). If a PI wishes to have a lab manager or administrative staff member given privileges to create and edit, or modify an on-line protocol, they must also attend a training session. Accounts are set up in conjunction with training sessions.

If you have any questions about this or want to schedule TOPAZ training, contact Jean Defendorf in the UCAR office (x5-1693).

Animal Use Categories, Definitions, and Examples

Category C:
Animals that will not experience pain, discomfort, or distress.

- Euthanasia using AVMA approved methods (including general anesthesia followed immediately by cervical dislocation or decapitation) for purposes of harvesting tissue, with or without fixation, in-situ.
- Behavioral observations.
- Natural breeding.
- Venipuncture for blood collection.
- Routine injections of non-toxic substances by IV, IP, SubQ, ID or IM routes.
- Genotyping using tail-snip without anesthesia (using the vivarium SOP) in preweanling mice only.
- Identification by ear punch or toe clip without anesthesia (using the vivarium SOP) in preweanling mice only.
- Tube feeding or gavage.
- Studies which use positive reinforcement or scheduled feeding or watering.
- Use of aversive stimuli that are mild, of limited duration, and can be avoided by the animal.
- Cervical dislocation or decapitation without the use of sedative, anesthetic or tranquilizing drugs as described in AVMA Guidelines on Euthanasia. Provide evidence that this method of euthanasia is scientifically justified, and that it will be done by specifically trained personnel using appropriate techniques and equipment.

Category D:
Animals that may experience pain, discomfort or distress but will be administered appropriate anesthetic, analgesic or tranquilizing drug to alleviate these effects.

- All major or minor recovery surgery.
- Procedures for which anesthesia or sedation is used, except euthanasia described in C above.

- Implantation of mini-osmotic pumps.
- Retrobulbar blood collection and intraocular injections under sedation, using DLAM procedures.
- Non-recovery surgical experiments (i.e.. assessing organ function followed by euthanasia).

Category E*:
Animals will experience pain, discomfort or distress for which anesthetics, analgesics or tranquilizing drugs would customarily be given but will not be administered because their use would adversely affect the interpretation of experimental results or interpretation.

- Tumor studies or monoclonal antibody production in which animals will experience pain not alleviated by analgesics.
- Tumor studies in which subjects exceed standard UCAR "end-points."
- Retrobulbar blood collection without sedation. Provide evidence that this procedure is scientifically justified and that it will be done by specifically trained personnel using appropriate technique.
- Exposure to radiation that produces clinical illness.
- Use of aversive stimuli that are unavoidable, such as inescapable electric shock or exposure to environmental extremes.
- Death as an endpoint.

***Note:** Federal regulations require that experiments conducted that are in this category must be specifically reported in the University's Annual Report to the United States Department of Agriculture. The Report must include the species, numbers and brief explanation of the scientific justification.

United States Department of Agriculture (USDA)

The United States Department of Agriculture (USDA) is a federal agency that promulgates and enforces federal regulations. The federal law is the Animal Welfare Act (Public Law 89-544) and amendments. A copy of the Animal Welfare Regulations is available for review in the UCAR office at x5-1693. The federal regulations define standards for the humane handling, care, treatment and transportation of dogs, cats, guinea pigs, hamsters, rabbits, nonhuman primates, and farm animals used in biomedical research or teaching. Nonregulated animals include laboratory bred species of rats (Rattus sp.) and mice (Mus sp.), poultry, birds, reptiles, amphibians and fish. The USDA requires that all research facilities be registered and report regulated animal use annually. The USDA reporting year is from October 1 through September 30. The annual reporting is required only for regulated animals (e.g., dogs, cats, rabbits, swine, sheep, goats, hamsters, guinea pigs, cotton rats, nonhuman primates, wild caught mammals).

USDA Animal Plant and Health Inspection Service (APHIS) veterinarians make unannounced inspections of research facilities. The inspector evaluates all aspects of the research facility's program for compliance with standards of the federal regulations. The inspector reviews protocols in the IACUC office, observes animals in the animal rooms and visits laboratories where animals are transported. Any items of noncompliance with the regulations are documented on the inspector's report and become available to the public

through the Freedom of Information Act. Serious items of noncompliance may place the research facility on probation or result in suspension of all regulated animal research activity. Please become familiar with the federal regulations. Please call the UCAR.secretary at x5-1693 to make an appointment with a UCAR member to gain more information on how the USDA Regulations impact your research.

USDA Animal and Plant Health Inspection Services (APHIS) Guidelines

Institutional Animal Care and Use Committee (IACUC) Guidelines

Note: These guidelines are not meant to replace the Animal Welfare Act (AWA), regulations or standards.

1. **Membership of IACUC: 2.31 (a)(b) and 2.33 (a)(3)**
    - 3+ members appointed by CEO. Includes at least one nonaffiliated person, one veterinarian, and a chair.

2. **Program Review and Facility Inspection: 2.31 (c)(1) and (c)(2)**
    - At least once every 6 months by at least two IACUC members

3. **Program Review and Facility Inspection Reports: 2.31 (c)(3)**
    - IACUC majority reviews, signs and submits to Institutional Official.
    - Contains minority view.
    - Identifies departures from regulations or standards and reasons.
    - Distinguishes significant from minor deficiencies.
    - Provides reasonable and specific plan and schedule and reasons.
    - Nonadherence to plan of correction for significant deficiencies reported within 15 business days to APHIS and any Federal funding agency.

4. **Review/Investigate Complaints About Animal Care: 2.31 (c)(4)**
    - Complaints may be from the public *or* from facility personnel [refer also to 2.32 (c)(4)].

5. **Recommendations Concerning Animal Care Program: 2.31 (c)(5)**
    - To Institutional Official about animal care and use program, facilities, or personnel training.

6. **Review of Protocols: 2.31 (c)(6) and 2.31 (d)(1-5) and (e)**
    - Review of ongoing protocols no less than once a year.
    - All IACUC members given list of proposed protocols prior to review.
    - Full committee (quorum) can be convened at request of *one* member.
    - IACUC reviewer must have no conflict of interest with protocol.
    - May use nonvoting consultants.
    - No study is to begin before IACUC review and approval.

7. **Review Significant Changes to Approved Protocol: 2.31 (c)(7) and (d)(8), 2.31 (e)**
    - IACUC must preapprove significant changes to protocol.

8. **Authorization to Suspend a Protocol: 2.31 (c)(8) and (d)(6), 2.32 (d)(7)**

- IACUC can suspend approved protocol with majority vote of convened quorum.
- Report suspensions to APHIS and any Federal funding agency.

9. **Field Study Exemption From Protocol and Faculty Review: 2.31 (c)(2) and (d)(1)**
   - Part 1 defines a field study as using free-living wild animals in a natural habitat without invasive procedures, harm or altering of behavior.

10. **Assurances and/or Statements Required in Written Protocols: 2.31 (d)(1) and (e)**. *All Proposed Animal Activities*
    - Procedure does not unnecessarily duplicate previous experiments.
    - Animals' living conditions are appropriate.
    - Medical care is available for the animals. (also 2.33)
    - Qualified and trained personnel are involved with procedures. (also 2.32)
    - Discomfort, distress and pain are minimized.
    - If used in two major surgeries, animal is not allowed to recover (unless justified).
    - Description of preoperative and postoperative care.
    - Survival surgery is conducted aseptically in dedicated facilities.
    - Rationale for species and number of animals.
    - Rationale for using animals.
    - Complete description of proposed use of the animals.
    - Complete description of procedures designed to assure that discomfort and pain are minimized.
    - Complete description of method of euthanasia.
      All Proposed Animal Activities With Potentially Painful Procedures 2.31 (d)(1)(i-iv)
    - Discomfort, distress and pain are minimized.
    - Method used, alternative information sources searched with a written list of parameters used for the search.
    - Procedure does not unnecessarily duplicate previous experiments.
    - Use of proper drugs for relief of pain/distress. No paralytics without anesthesia.
    - Attending veterinarian is involved in planning. (also 2.33)

11. **IACUC Records: 2.35 (a)(1-3)**
    - Minutes, proposed activities or significant changes, approvals or disapprovals of protocols, and semiannual reports with recommendations.

12. **Exceptions to Regulations and Standards: 2.36 (b)(3)**
    - IACUC-approved exceptions to regulations and standards attached to annual report (APHIS Form 7023).

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information

(braille, large print, audiotape, ect.) should contact the USDA Office of Communications at (202) 720-5881 (voice) or (202) 720-7808 (TDD).

To file a complaint, write Secretary of Agriculture, U.S. Department of Agriculture, Washington, DC 20250, or call (202) 720-7327 (voice) or (202) 720-1127 (TDD). USDA is an equal employment opportunity employer.

New York State Department of Health (NYSDH)

Animal research facilities must be licensed by the New York State Department of Health. The license is renewed annually. The New York State Department of Health Inspector makes unannounced visits and evaluates the care and use of all live vertebrate animals (mammals and birds) used or intended for use in biomedical research, teaching or testing. The State Inspector examines the animal care and research program and facility for compliance with the USDA Regulations, State Regulations and the PHS Policy on the Humane Care and Use of Laboratory Animals and Guide for the Care and Use of Laboratory Animals. The State Inspector may visit the animal rooms, inspect the researcher's lab and review UCAR protocols. Any deficiencies are stated with deadline for correction in a written report. Serious deficiencies may result in revocation of the facility's license and suspension of animal research activity.

Public Health Service (PHS)

The Public Health Service (PHS) is a federal agency that includes the National Institutes of Health (NIH). NIH is a funding agency that supports research activities at qualified institutions. An investigator qualifies for NIH funding only if there is an institutional Assurance on file with the Office of Laboratory Animal Welfare (OLAW). The Assurance describes how PHS funded animal activities are performed in compliance with the PHS Policy on Humane Care and Use of Laboratory Animals and the PHS Guide for the Care and Use of Laboratory Animals (Guide). The PHS Policy and Guide state principles of care and use of any vertebrate animal in a PHS funded study. The letter of Assurance describes the Institutional Program for the Care and Use of Animals and the Institutional Animal Care and Use Committee. The Institutional Program for the Care and Use of Animals includes lines of authority for administering the program and the authority, qualifications and responsibility of the attending veterinarian. A description of the training program available to scientists, research technicians and animal care technicians is also required. The PHS Policy and Guide are used as basis for the program formation and description. The PHS Guide states requirements and recommendations for institutional policies for personnel qualifications, occupational health, aseptic surgery, laboratory animal husbandry, veterinary care, and the physical plant. The assurance is updated annually and considered for renewal every five years. Investigators and staff are encouraged to be familiar with all aspects of the written Assurance. Please read the request from OLAW. The Assurance, the Guide and the PHS Policy are available for review in the UCAR office (x5-1693). Please call the UCAR secretary to schedule a meeting with a UCAR member for more information about PHS conditions of funding protocols.

OLAW Reports

January 11, 1994
Subject: Internal Distribution of Your Animal Welfare Assurance
Dear Colleague:

This letter is being forwarded to Institutional Officials and Institutional Animal Care and Use Committee (IACUC) Chairs throughout the country. Its purpose is to convey a recommendation to all institutions conducting animal-related activities supported by the U.S. Public Health Service (PHS).

The Animal Welfare Assurance (Assurance) represents a legally binding institutional commitment to the PHS, necessary for eligibility to receive PHS support. Because of its fundamental importance to that relationship, and because it outlines the mechanisms for implementation of the PHS Policy on Humane Care and Use of Laboratory Animals (Policy) at the institution, the Office for Protection from Research Risks (OPRR) believes that the core contents of the Assurance should be generally known by all interested parties at the institution. Although IACUC members usually have some knowledge of the document, OPRR has encountered situations where individual members had not actually seen it. More frequently, many investigators and their staffs have expressed a complete lack of awareness of the document.

When assessing the compliance with the PHS Policy by awardee institutions, e.g., during site visits, one of the principal standards against which institutional performance is measured is the extent to which the Assurance is adequate and accurate. In other words, does the Assurance conform with the basic requirements of the PHS Policy and is it and accurate description of the actual practices of the institution? These same questions should always be addressed by the IACUC during the semiannual review of programs and facilities. Major discrepancies between described and actual practices have been the basis for a number of adverse findings and actions by OPRR.

Therefore, OPRR recommends that the institution make the core contents of their Assurance widely available within the institution for the information purposes and as an educational tool, not only for IACUC members, but also for animal care staff, investigators, administrators, and other interested parties.

Thank you for your attention to this important matter. Please contact the professional staff of the division of Animal Welfare, OPRR (301/496-7163), if you have any questions concerning this or any other PHS Policy matter.

Sincerely,

Gary B Ellis, Ph.D.
Director, Office for the Protection from Research Risks

Nelson L. Garnett, D.M.V.
Director, Division of Animal Welfare, OPRR

National Institutes of Health, Building 31, Room 5B63, 9000 Rockville Pike, Bethesda, MD 20892

Chapter 2: Biomethodology of Laboratory Animals

Anesthesia

Pain: Recognition of pain depends upon intact pathways from pain receptors to the thalamus and cerebral cortex, as well as functional cerebral cortex and subcortical structures. Thus any means that renders the cerebral cortex nonfunctional, such as hypoxia or drug depression, prevents pain. When this happens, stimuli that evoke motor nerve reflexes that may be painful to the conscious animal are not painful in the unconscious animal. Equally painful stimuli administered to animals chemically paralyzed by curare or succinylcholine will not evoke a motor reflex simply because of paralysis, but will cause pain because of the conscious state. Hence, it is possible that unconscious animals may feel no pain but respond to certain stimuli, and paralyzed animals may feel pain but cannot respond. Thus, movement is not a reliable indicator of pain, and paralyzing agents (i.e., succinylcholine and curare) are strictly prohibited as euthanatizing agents. The methods used for prevention or relief of pain and distress in scientific experimentation with living animals will be dependent upon the kind of procedures used on the animals. Selection of an appropriate anesthetic, analgesic, or tranquilizer require the assistance of an experienced professional.

Anesthesia:

The definition is the local or general loss of sensation. General anesthesia is achieved by depressing the brain receptors of pain, thus producing a general anesthetic effect, although not necessarily blocking local responses such as spinal cord reflex arcs. Therefore, it is possible to have good levels of general anesthesia but still have motor reflexes such as pinch-pad and corneal reflexes present. These should not be mistaken for purposeful responses to pain. They can however, be abolished by deepening the level of anesthesia. Great care must be exerted when general anesthesia is made too deep since not only are pain receptors depressed, but also the vital centers of the brain and brain stem including respiratory, cardiac, hypothalamic, etc. When depressed for too long, heart and respiratory function cease and death ensues unless heroic measures are taken--if they are available.

Regardless of the species involved, some principles of general anesthesia are universal and worth keeping in mind. They include:

A. **Maintain patent airway.** This is essential if trouble arises and the subject is to survive. Nothing must block the ability to breathe freely and easily. With small rodents that are obligate nose breathers, a patent airway is easily maintained if the nostrils are not blocked.
B. **Avoid hypothermia.** Core body temperature can fall alarmingly, particularly in small animals, during the course of prolonged general anesthesia. Hypothermia added to other factors can produce an irreversible sequence of events leading to death. Thermostatically controlled heating pads should always be used in animal surgery.
C. **Administer anesthetic to effect.** Technically, because of wide variation within and between species, there is no such thing as predetermined anesthetic dose of a drug. General anesthesia must be given to effect, as measured by physiological

parameters and response to stimuli. Most anesthetic deaths can be attributed to not following this principle. This is especially true for parenterally administered drugs such as barbiturates. Once they are injected, there is little the anesthetist can do to control the outcome; therefore, great care is necessary when administering these drugs. Most methods of general anesthesia as listed in Table 1 are generally safe and reliable if properly administered and monitored.

Drug Dosage – Anesthesia and Analgesia - Please review to the Sedation/Tranquilization, Anesthesia and Analgesia in Laboratory Animals and Veterinarian-Recommended Formularies or contact a DLAM veterinarian (X5-2653).

Criteria for the Administration of Analgesics in Laboratory Animals

RODENT ANALGESIA ...pain in rodents may be identified by observing the animal's reluctance to move about, eat or drink, weight loss, salivation, hunched posture, piloerection, respiratory sounds (chattering in mice) and by vocalization with handling.

GUINEA PIG AND CHINCHILLAS..pain in guinea pigs and chinchillas may be identified by observing the animal's reluctance to move, vocalization with handling, decreased food and water intake and postural abnormalities.

FERRET ANALGESIA..pain in ferrets may be identified by observing the animal's reluctance to move, spontaneous vocalization or vocalization upon handling, reluctance to eat and drink, avoidance behavior, depression, postural abnormalities, increased respiratory rate and abnormal pattern.

RABBIT ANALGESIA...pain in rabbits may be identified by observing the animal's reluctance to move about, eat or drink, postural abnormalities, increased respiratory rate and/or abnormal pattern and by vocalization with handling.

NONHUMAN PRIMATE ANALGESIA..pain in nonhuman primates may be identified by depression, guarding of painful part, avoidance behavior, spontaneous vocalization or vocalization upon handling, teeth grinding, lying down and getting up repeatedly, abnormal posture, increased respiratory rate and abnormal pattern, reluctance to move or inappetence.

CANINE ANALGESIA..pain in dogs may be identified by depression, guarding of painful part, spontaneous vocalization upon handling, avoidance behavior, recumbency, inappetence, muscle tremors, attraction to painful area (licking, biting, scratching), and abnormal posture.

SWINE ANALGESIA..pain in pigs may be identified by depression, recumbency, vocalization when painful area is manipulated or spontaneous vocalization (e.g. grunting), abnormal posture, inappetence, increased respiratory rate and abnormal pattern, lying down and getting up repeatedly and avoidance behavior.

SHEEP/GOATS..pain in sheep and goats may be identified by depression, recumbency, vocalization when painful area is manipulated or spontaneous vocalization, abnormal posture, inappetence, increase respiratory rate and/or abnormal pattern, lying down and getting up repeatedly (especially in ruminants) and avoidance behavior.

FELINE ANALGESIA..pain in cats may be identified by depression, guarding of painful part, spontaneous vocalization or vocalization upon handling, avoidance behavior, loss of appetite, reluctance to move, abnormal posture, muscle tremors, and attraction to area of pain (licking, biting, scratching).

Analgesia

Analgesia is insensitivity to pain without loss of consciousness. This is a general effect and involves depression of brain receptors as well as brain centers. A variety of drugs have analgesic properties when given in the proper dosage. Some categories of drugs do not produce analgesia, therefore, a list of commonly used terms is provided below for clarification.

A. **Analgesic:** Drugs like morphine, meperidine (DemerolR) and codeine which alleviate pain without causing a loss of consciousness.
B. **Anesthetic:** A drug or agent that is used to abolish the sensation of pain. Sodium pentobarbital, when injected intravenously or intraperitoneally, depresses the central nervous system and induces deep sleep during which the sensation to pain is lost.
C. **Cataleptic:** A drug like ketamine hydrochloride which produces a trance-like state of hyporesponsiveness which is known as dissociative anesthesia. Because of the nature of its activity, ketamine does not produce analgesia for pain which accompanies abdominal, thoracic or CNS surgery or manipulation of fractured bones. In the latter cases, a tranquilizer or sedative must be used in conjunction with ketamine.
D. **Sedative:** An agent which allays activity and excitement by producing a mild degree of central nervous system depressing in which the patient is awake but calm and free of nervousness. Xylazine (RompunR) acts as an analgesic and a sedative but it is not a tranquilizer or an anesthetic.
E. **Tranquilizer:** Drugs like promazine, acetylpromazine, and diazepam (ValiumR) act on the emotional state to calm and quiet the patient. These drugs increase the threshold to environmental stimuli and depress many physiological functions but do not produce sleep, analgesia or anesthesia. When used in combination with dissociative anesthetics, a degree of general anesthesia is effective for certain and procedures in small laboratory animals.
F. **Narcotic:** Any of a class of addictive substances, such as opium and morphine, that blunt or distort the senses and in large quantities produce euphoria, stupor or coma.

Table 1 lists anesthetics or analgesics that have been successfully used in rodents and birds. More detailed information on anesthesia and anesthesia equipment is available from DLAM.

NOTE: Barbiturates are caustic substances when injected into living tissue and care must be taken to completely avoid subcutaneous or intramuscular injection with these drugs. Intravenous injection is preferred and intraperitoneal injection is acceptable with diluted material.

**In the event that the analgesic described in the UCAR protocol is unavailable, please contact a DLAM veterinarian so they may provide a satisfactory substitute and oversight of the change in analgesic therapy.*

Euthanasia

Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline levels of cellular or extracellular biological values must be minimized.

Euthanizing agents terminate life by one of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of brain tissue. Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful.

When using these or any other method of euthanasia, it is important to take adequate measures to insure animals are dead and have no chance to revive or regain consciousness at any later time, especially when using anesthetics.

All methods must be recommended by the AVMA Guidelines on Euthanasia: 2013 Edition.

You may use decapitation or cervical dislocation of rodents without prior sedation/analgesia, but must provide a scientific justification for doing so. For example, you can provide a statement that analgesia/sedation will affect the end point measurements you make. You should also provide a reference supporting your justification. In addition, you must provide a statement that the persons performing the euthanasia technique are appropriately trained and are using proper equipment. You should specifically indicate who will be performing euthanasia in the Associates section of the protocol.

Procedures such as CO2 euthanasia or anesthetic overdose on rodents, where death may not be immediately evident, must include a secondary physical method for ensuring death. These include decapitation, pneumothorax by opening the thoracic cavity, cervical dislocation of rodents under 200 g, complete severance of the spine just below the base of the skull using a dorsal approach, or perfusion of a histological fixative via the major blood vessels.

There are special considerations for euthanizing rodent embryos, fetuses and neonates. Please see the UCAR policy on Euthanasia for Rodent Embryos, Fetuses and Neonates on the UCAR website.

Approved Euthanasia Dosage and Techniques for Rodents

1. Carbon Dioxide Inhalation Chamber followed by secondary physical method (i.e. pneumothorax, cervical dislocation for rodents under 200 grams, decapitation, perfusion of a histological fixative via the major blood vessels or complete severing of the spine just below the base of the skull using a dorsal approach)
2. Surgical plane of anesthesia followed by a physical method. This includes perfusion with a fixative or exsanguination. Any anesthesia approved for surgery may be used, but the depth of anesthesia must be verified before the secondary method is applied.
3. Anesthetic overdose followed by a physical method. Any AVMA (AVMA Guidelines on Euthanasia: 2013 Edition) approved anesthetic overdose is acceptable (e.g. barbiturate or euthanasia solution 100 mg/kg IV or IP or a combination of Ketamine 300-360 mg/kg + xylazine 30-40 mg/kg IP.

4. Cervical dislocation for rats weighing less than 200 grams and all mice after sedation (unless otherwise scientifically justified to U.C.A.R.)
5. Decapitation with guillotine only after the animal has been sedated (unless otherwise justified to U.C.A.R.)

Reference

Schoell et al (2009). Euthanasia Method for Mice in Rapid Time-Course Pulmonary Pharmacokinetic Studies –
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2755020/pdf/jaalas2009000506.pdf

Reviewed and updated 2-20-16 eh

Housing – Rodents

MOUSE CAGE DENSITY POLICY

Overcrowded mouse cages represent a significant animal welfare concern. Such cages are noncompliant with Public Health Service (PHS) Policy and our Assurance to PHS. *The Guide for the Care and Use of Laboratory Animals* states the PHS recommendations for housing densities. In order to standardize housing densities and prevent or eliminate the possibility of overcrowding within cages, the University's Animal Resource has adopted the following UCAR-approved policy. **Ventilated cages accommodate up to five mice greater than 28 days of age. Static cages accommodate four mice greater than 28 days of age.** Cage densities exceeding these numbers represent clear policy violation.

Breeding

- Standard Breeding: 1-3 females: 1 male; pregnant females housed individually before birth of pups
- Continuous Breeding: 1 male: 1 female; not separated before birth of pups. This strategy takes advantage of the post-partum estrus which occurs within 14 -28 hours after parturition.

- The breeding strategy utilized must be described in the UCAR protocol.

- Justification is required for continuous breeding and/or for cage densities which exceed those described above.

- Continuous trio breeding results in overcrowded cages (e.g. male and two females and litter(s)) which must be cleaned more frequently. For that reason, this strategy is discouraged and requires scientific justification. If necessary (and justified and approved by UCAR), PIs must submit a special request describing the continuous trio breeding strategy and obtain cage cards/stickers that identifies cages as continuous trio breeders. These cages are subject to a higher per diem associated with more frequent cage changes which are required for these higher density cages.

Weaning

Investigators who choose to manage their own breeding colonies are responsible for timely weaning. Most mice may easily be weaned at 21 to 23 days of age, but this period may be extended to 28 days for fragile mice. The Animal Resource staff reports the date new pups are found on the cage card. Litters not weaned before their 29th day of age will be reported to the investigator on day 29 with a requirement for the PI or staff to wean that day. DLAM will separate unweaned litters the following morning for a charge of $50.00 per cage. If the investigator weans

the cage but fails to inform DLAM, a fee will be charged. Delayed weaning protocols must be approved by UCAR with specification of actual weaning ages (up to 35 days of age) for extremely fragile mice. Additionally, a special request must be submitted to the Animal Resource office identifying the group of mice approved for delayed weaning. Continuous breeding with delayed weaning is not permitted.

If a breeding strategy results in two litters from the same dam, the older litter must be weaned when the new litter is born. The researcher has 24 hours to wean the older litter to prevent an overcrowded cage. If DLAM reports the cage for having two litters, the researcher has 24 hours to wean the older litter, and must contact DLAM to report that the litter has been weaned. If this is not done, the cage will be counted as overcrowded. This means that litters may require weaning between 17 and 20 days to prevent overcrowding and trauma to newborn pups. The investigator is expected to provide supportive care (moistened chow, Hydrogel®, small house in vent racks) and daily observation until early weaned pups are self-sustaining. If the younger litter is being harmed by the older pups, DLAM will immediately wean the older litter for a fee and notify the investigator.

The DLAM veterinary staff provides training in the management of rodent breeding colonies for investigators and their staff. DLAM also offers colony management services to PIs for a fee.

Overcrowded Cages

Overcrowded cages (> five mice over 28 days of age in ventilated cages, > four mice over 28 days of age in static cages) will be reported to investigator. DLAM will remove mice from overcrowded cages if the investigator has not done so by the day following notification. There is a fee for this service.

Identification

A completed cage card must be present on all mouse cages. Please refer to the Animal Resource website (http://www.urmc.rochester.edu/vivarium/Barcoding.cfm) for information on cage card activation. The information on the card should include: the investigator's name, the approved UCAR protocol number, an animal identification number (if applicable), the mouse strain/stock and the account number. Individual animal identification such as ear punches, ear tags, toe clips, tattoos and implantable transponders is encouraged, especially in cases where animals are group housed and/or appear identical. All methods of identification must be described in the animal protocol and approved by UCAR.

RAT CAGE DENSITY POLICY

Overcrowded rat cages represent a significant animal welfare concern. Such cages are noncompliant with Public Health Service (PHS) Policy and our Assurance to PHS. The *Guide for the Care and Use of Laboratory Animals* states the PHS recommendations for housing densities. In order to standardize housing densities and prevent or eliminate the possibility of overcrowding within cages, the University's Animal Resource has adopted the following UCAR-approved policy**:** **The number of rats per cage depends on the weight of the rat(s) and the size of the cage**.

Required Floor Space Per Rat

A standard rat cage has143 square inches floor space.

<100 g per rat = 17 sq inches = 8 rats
Up to 200g per rat = 23 sq inches = 6 rats
Up to 300g per rat = 29 sq inches = 4 rats
Up to 400g per rat = 40 sq inches = 3 rats
Up to 500g per rat = 60 sq inches = 2 rats
> 500g per rat = >70 sq inches = 1 rat

Female w/ litter = 124 sq inches

Breeding

- Standard breeding: 1-3 females:1 male. Pregnant females are housed individually before birth of pups.

- Continuous breeding: 1 male:1 female; not separated before birth of pups. This strategy takes advantage of the post-partum estrus which occurs within 14-28 hours after parturition.

- The breeding strategy utilized must be described in the UCAR protocol.

- Justification is required for continuous breeding and/or for cage densities which exceed those described above.

- Continuous trio breeding results in overcrowded cages (e.g. male and two females and litter(s)) which must be cleaned more frequently. For that reason, this strategy is discouraged and requires scientific justification. If necessary (and justified and approved by UCAR), PIs must submit a special request describing the continuous trio breeding strategy and obtain cage cards/stickers that identify cages as continuous trio breeders. These cages are subject to a higher per diem associated with more frequent cage changes which are required for these higher density cages.

Weaning

Investigators who choose to manage their own breeding colonies are responsible for timely weaning. Rats are generally weaned at 21 days of age. At this age, the pups are placed on inventory by the vivarium staff and the PI is notified. At 23 days of age, the PI will be notified if litters have not been weaned. The following day, these rats will be weaned by DLAM for a $50.00 fee. Delayed weaning protocols must be approved by UCAR with specification of actual weaning ages (up to 28 days of age) for extremely fragile rats. Additionally, a special request must be submitted to the Animal Resource office identifying the group of rats approved for delayed weaning. Continuous breeding with delayed weaning is not permitted.

If a breeding strategy results in two litters from the same dam, the older litter must be weaned when the new litter is born. This means that litters may require weaning between 17 and 20 days to prevent overcrowding and trauma to newborn pups. Cages identified with two or more litters will be reported to the PI. If the younger litter appears to be unaffected by the presence of the older pups, the investigator must wean the older litter by the following day. If not, DLAM will wean the older litter for a fee. If the younger litter is being harmed by the older pups, DLAM will immediately wean the older litter for a fee and notify the investigator. The investigator is expected to provide supportive care (moistened chow +/- Hydrogel®) and daily observation until early weaned pups are self-sustaining.

The DLAM veterinary staff provides training in the management of rodent breeding colonies for investigators and their staff. DLAM also offers colony management services to PIs for a fee.

Overcrowded Cages

Cages containing rats which exceed the floor space requirements are considered overcrowded. These cages will be reported to investigators. DLAM will remove rats from overcrowded cages if the investigator has not done so by the day following notification. There is a fee for this service.

Identification

A completed cage card must be present on all rat cages. Please refer to the Animal Resource website (http://www.urmc.rochester.edu/vivarium/Barcoding.cfm) for information on cage card activation. The information on the card should include: the investigator's name, the approved UCAR protocol number, an animal identification number (if applicable), the rat strain/stock and the account number. The use of individual animal identification such as ear punches, ear tags, tattoos or implantable transponders is encouraged, especially in cases in which animals are group housed and/or appear identical. All methods of identification must be described in the animal protocol and approved by UCAR.

Environmental Enrichment and Social Housing – Rodents

Rodent Enrichment Policy

Background

The primary aim of environmental enrichment is to:

I. Enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources;

II. Facilitate the expression of species-typical behaviors; and

III. Promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics.

Scope

This policy applies to all rodents housed at the University of Rochester.

Policy

Each animal should be provided with the opportunity to exhibit species typical behavior. Primary enclosures are continually evaluated by the Department of Laboratory Animal Medicine (DLAM) to provide laboratory rodents with an appropriate environment that enhances their well-being. *Social housing will be considered as the default method of housing social rodents* unless otherwise justified based on social incompatibility resulting from inappropriate behavior, veterinary concerns regarding animal well-being, or scientific necessity approved by the UCAR. *Environmental enrichment for rodents will be considered the default unless scientifically justified and approved by UCAR (see below for species specific enrichment strategies).*

Procedures

I. Basic structural and environmental components

A. Cages with solid bottoms are the default to provide a comfortable resting and walking surface. Investigators must scientifically justify the use of novel housing, metabolic cages and wire-bottom cages. When approved by UCAR, rodents may be

housed on wire-bottom cages for the minimum period required to achieve the scientific goals of the study.

When group housed rats exceed the minimum floor space requirements in standard rat cages, they are moved into larger cages to allow them to maintain social housing. Alternatively, UCAR may exempt animals from the minimum space requirements to allow social housing. In these cases, animals will be monitored, and changes in husbandry (i.e., frequency of cage changes), may be implemented to ensure the overall well-being of the animals.

B. All rodents are provided with bedding to allow for species-typical burrowing and nest building. Corn cob bedding (Bed-o'cobs, Andersons Lab) is the default, but investigators may request alternative beddings if necessary.

C. In addition to structural elements, all rodents will have at least one environmental enrichment item added to the cage. Environmental enrichment typically used for rodents includes pads of compressed bedding (Nestlets®), nesting sheets, long strands of crinkled paper (Crink'l Nest®), and/or polycarbonate shelters (Mouse house). Additional products may include Alpha-Dri Plus (pre-enriched bedding), Shepherd shacks, plastic huts or houses, and/or plastic pipes. These products block ambient light and encourage nest building activities, provide sheltered areas, or control temperature within the primary enclosure.

D. Polycarbonate tubes for tunneling, rat lofts, and Nylabones® for gnawing may also be provided to rats.

E. Enrichment practices by species:

1. Mice: Social housed animals will receive nesting material.
2. Rats: All rats will receive nesting sheets and polycarbonate tunnels.
3. Gerbils & Hamsters: Nesting material and a shelter.
4. Naked Mole Rats: Complex, multi-chambered housing unit.
5. Blind Mole Rats: Deep bedding for burrowing.
6. All single housed rodents will receive enhanced enrichment.

II. Nutritional Enrichment

The use of species appropriate diet or dietary supplement items may be used as a means of enrichment. Food treats may be provided to rodents providing they do not significantly reduce the consumption of the normal diet (ex. Veggie Relish; LabDiet, 5LRV, irradiated).

III. Rodents Requiring Special Attention

Occasionally, rodents exhibit behavior that suggests they may benefit from a more complex environment. If DLAM determines that additional enrichment is required for any rodent (e.g. self-inflicted lesions, continuous wire gnawing, jumping, circling,

tail-chasing, excessive food shredding), manipulata such as Nylabones, cardboard tubes, mouse houses and/or tunnels will be provided.

IV. Exemptions

UCAR recognizes the following exemptions to the requirement for social housing:

1. Scientific Exemptions
    a. Investigators must scientifically justify single housing in the Protocol, and UCAR must approve this exemption
    b. UCAR will carefully examine the justification, and may require that animals on certain studies in a Protocol be socially housed while recognizing that other experiments within the same Protocol may require single housing
    c. In all cases, single housing will be for the minimum period required to achieve the scientific goals

2. Veterinary Exemptions
    a. Injured, ill, or debilitated rodents may be single housed at the discretion of DLAM
    b. Female rodents which barber subordinate animals to the point where they cause skin lesions in subordinates may also be exempted

3. Social Incompatibility
    a. Male rodents are exempt from social housing requirements once they have been removed from the cage where they are first housed with other males
    b. Syrian hamsters are exempt from social housing requirements once they have been removed from the cage where they were first housed with others of their own gender
    c. Blind mole rats

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Handling of Common Laboratory Animals

Mice: Mice are usually caught and lifted by the tail. The tail should be grasped about two-thirds of the way down. With this simple method of holding, they may be transferred to another cage or a balance, identified or sexed; but such restraint is not sufficient for treatment and close examination. For more effective control, the mouse may be held by the tail and placed on a table or other surface, preferably one that the mouse can grasp, and the loose skin over the neck and shoulders grasped with thumb and fingers. In the process of grasping at this point, the mouse can turn and bite, but once grasped correctly, the head is adequately controlled. With the tail and rear legs held by other fingers or the other hand, a good hold for re-examination or treatment is possible (Figure 1).

Figure 1: Manual Restraint of the Mouse

Rats: Rats quickly become conditioned or trained to tolerate routine and frequent handling. Rats are normally lifted by grasping the whole body--palm over back and side with forefinger behind the head and the thumb and second finger in opposite axial extending the forelimbs so that they may be controlled (Figure 2). Rats may also be temporarily restricted by the base of the tail. Holding with one hand is usually adequate for control, but tail, rear legs or lower part of the body may be held by a second person. Young rats may be handled in a way similar to that for mice, when body size does not permit ease of handling with one hand. Rats will bite, and certain strains are more aggressive than others. Various restraining devices are available for use with rats. Check with DLAM for assistance and instruction.

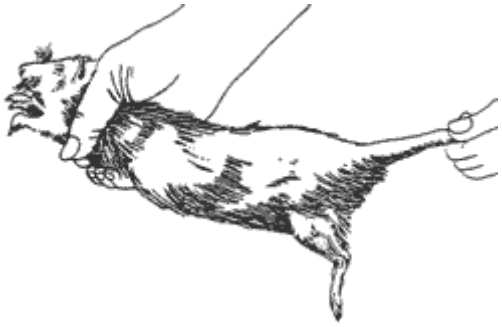


Figure 2: Manual Restraint of the Rat

Hamsters: Hamsters will bite quickly and deeply and are easily aroused, consequently they should be approached gently and with caution until they become accustomed to being handled and familiar without the handler. Several methods may be useful in handling the hamster. Both hands may be cupped under the animals to hold in the palms. They may be picked up with one hand, in a similar manner to that of the rat. Grasping the loose skin over the neck and shoulder also provides an effective method of control with one hand; however, this skin is very loose and practice is necessary before this method can be used casually (Figure 3). It is sometimes easier for the occasional handler to use a cup when transferring hamsters from point to point when fine manipulations are not necessary.

Figure 3: Manual Restraint of the Hamster

Gerbils: Gerbils respond to and are effectively handled by the general methods indicated for other rodents. Lifting by the base of the tail near the body is desirable. Avoid holding the gerbil near the end of the tail since the skin near the tip of the tail is fragile and may slip off.

Guinea Pigs: Guinea pigs seldom bite but are timid or easily frightened and usually make determined efforts to escape when held. They are best held by placing the thumb and forefinger around the neck with the palm over the back under the abdomen and the other fingers grasping the body. When lifting, the other hand should be used to support the lower part of the body (Figure 4). Special care should be exercised in handling pregnant females, since they may become very heavy and awkward.

Figure 4: Manual Restraint of the Guinea Pig

Rabbits: Rabbits seldom bite but can inflict painful scratch wounds, especially with the hind feet. Hold them in a way that directs their hind feet away from your body. Grasping the loose skin over the shoulder with the head directed away from the holder is the best method of initial restraint. When lifting, the lower part of the body must be supported by the other hand (Figure 5). Rabbits should never be lifted by the ears or the neck. If the rabbit begins to struggle violently and develops rotational movements with the hindquarters, it should immediately be placed on a solid surface and calmed. Continued violent struggling frequently leads to fracture of one or more lumbar vertebrae and fatal injury to the spinal cord. Particularly important are mechanical restraints such as the one shown in Figure 6. These are necessary for safely restraining rabbits for most procedures. Practice in using these devices can be arranged through DLAM.

Figure 5: Manual Restraint of the Rabbit

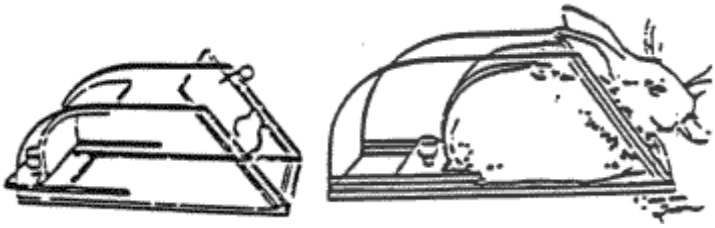


Figure 6: Mechanical Restraint of the Rabbit

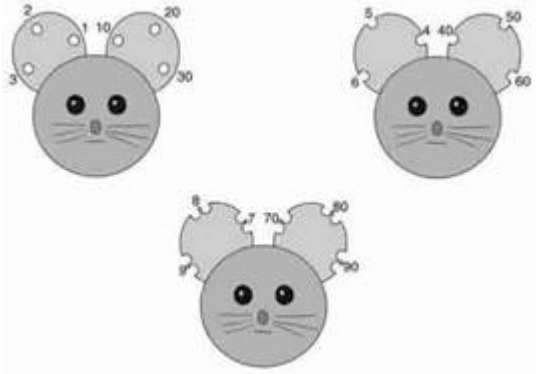
Please contact DLAM for techniques in handling larger laboratory animals such as nonhuman primates, dogs, cats and swine. All nonhuman primates must be tranquilized with ketamine hydrochloride (10 mg/kg IM) for handling unless chair restraint is used with aid of a collar and leash.

Rodent Identification Methods

There are several acceptable methods to permanently identify laboratory rodents. A description of the identification method used must be included in answer Section A #15 of your approved University Committee on Animal Resources (UCAR) protocol.

EAR PUNCH: This is a commonly used procedure which employs a special metal punch instrument to place a hole in the ear of the rodent, following a code (below). **Advantages:** (1) quick and easy to perform (2) inexpensive (3) relatively atraumatic (4) no anesthesia required (5) punched tissue can be used for DNA (PCR) screening. **Disadvantages:** (1) cannot be performed on pups under two weeks of age due to size and position of ears (2) potential for ear damage (3) may be difficult to read.

Ear punch numbering system



EAR TAGGING: Numbered metal clips can be applied to the base of the pinna with special pliers. Various sized tags exist; the appropriate size must be selected for the

species being identified. **Advantages:** (1) quick and easy to perform (2) relatively atraumatic (3) no anesthesia required (4) relatively inexpensive. **Disadvantages:** (1) cannot be performed on pups less than three weeks of age due to size and weight of tags (2) tags can fall out (3) tags may cause granulomas at site of application.

TOE CLIPPING: This method involves the removal of the distal portion of no more than one toe per foot and no more than two feet per individual animal may be toe clipped. The Guide for the Care and Use of Laboratory Animals states that this identification method is only appropriate for altricial neonates and is to be used when a less invasive method of identification is not practical. Because this method may cause more than momentary pain, its use must be scientifically justified and approved by UCAR. Toe clipping must be performed in accordance with the UCAR Toe Clipping Policy. **Advantages:** (1) easy to read (2) inexpensive (3) can be successfully employed in neonates (4) clipped tissue can be used for DNA (PCR) screening. **Disadvantages:** (1) may cause pain, (2) lameness, (3) infection and (4) decreased grasping ability.

TATTOOING: Tattoo ink can be injected under the skin of all rodents, using either a tattoo needle or a hypodermic needle and syringe. Appropriate tattoo sites include: tail –all rodents, ears –guinea pigs. Neonatal rodents may be tattooed on the ear, tail, hock or toe. **Advantages:** (1) easy to read (2) can be used on neonates. **Disadvantages:** (1) requires anesthesia (2) may require special equipment (3) potential for infection (4) tattoos can fade or spread as the animal ages (5) may be difficult to read in pigmented animals.

ELECTRONIC TRANSPONDERS: microchip transponders are implanted via subcutaneous injection. A special recording instrument reads and displays the number on the scanner. **Advantages:** (1) no anesthesia required (2) easy to read (3) quick placement of chips (4) some chips can be linked to computer system that records other data about the animal. **Disadvantages:** (1) initial cost of equipment (2) chips can fall out (3) requires special equipment to read identification (4) potential for infection.

DLAM Mouse Tail Biopsy SOP

PURPOSE

To provide instruction for obtaining genetic material for DNA isolation via tail biopsy.

MATERIALS

Identification instrument
Straight edged razor blades – 1 blade per 2-3 animals
Autoclaved nestlet or paper towels
Specimen vials (e.g. eppendorf tubes)
Tail biopsy log
Anesthetic (for older rodents)
Styptic or antibiotic powder (for older rodents)

PROCEDURE

1. Tail biopsies may be performed on mice or rats of any age. Anesthesia (e.g. ketamine or isoflurane) is required for rodents beyond weaning age. Inject ketamine (diluted 1:10 with sterile saline or water for injection for mice) at a dosage of 60-90 mg/kg intraperitoneally or induce with 3-4 % Isoflurane in a chamber at flow rate of 2 L/min.

2. Identify animals by ear punch, ear tag, etc. as described in the approved UCAR protocol
3. Manually restrain the mouse/rat with distal portion of tail situated on surface of nestlet or paper towel.
4. Using 1/3 of a straight edged blade, remove ~ 4 mm of distal tail. Some bleeding will occur. For animals of weaning age, apply slight pressure to the tip of tail until bleeding stops. Heavier bleeding is more likely in older mice and rats. For this reason, it is recommended to dip each tail in styptic powder or an antibiotic powder (e.g. Biozide).
5. Return rodent to cage.
6. Place tail tissue in specimen vial and label with animal ID number. If you are maintaining a tail biopsy log, be sure to record animal id, strain, cage #, dob, gender and parent info on the log sheet when applicable.
7. The unused part(s) of the blade can be used to transect the tail tip on the next animal, making certain to avoid any blood contamination. Once each part of the blade has been used once (2-3 mice), discard the blade in a sharps container.
8. Change nestlets or paper towel when they become soiled, or between investigator's animals.
9. Store specimens in a designated freezer.

Toe Clipping

Toe-clipping is one of several permanent methods of identification used on mice, rats and birds. It involves the removal of the phalangeal bone from the most distal joint to the tip of certain toes with a sharp instrument, according to a numbering code. The toe-clipping procedure is considered potentially painful and may impair an animal's ability to grip and groom. According to the Guide for the Care and Use of Laboratory Animals, "Toe-clipping, as a method of identification of small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates."

The University Committee on Animal Resources (UCAR) recognizes that under certain circumstances, it may be necessary to use toe clipping as a method of identification in mice, rats and birds. In accordance with the Guide, UCAR has established the following toe-clip policy:

1. Strong scientific justification for the toe-clip procedure must be provided in the protocol and approved by UCAR. The justification should include a discussion of alternate identification methods (e.g. ear punch, ear tag, tattoo, leg bands in birds) that the investigator has considered and the reason why such methods are unsatisfactory. For example, an investigator may justify toe clips if his/her research requires permanent marking of rodents genotyped at a young age. Toe-clipping can also be considered a refinement if genetic material for analysis can be obtained at the same time as the rodents are identified, therefore making it unnecessary to perform tail biopsies for tissue sampling. For birds, toe-clipping in combination with daily records identifies the bird and its hatch day until the chick is large enough to be banded at post hatch day (PHD) 10 to 15. Banding sooner can injure the chick or result in a loss of the band.

2. In the event that an acceptable alternative method of identification becomes available, the investigator should consider the feasibility of using the new method as a replacement for toe- clipping.
3. Toe-clipping is only approved under the following conditions:

    - The procedure MUST be performed no later than seven (7) days of age in mice and rats, and no later than three (3) days post hatching in birds.
    - No more than two toes per foot may be clipped in mice and rats. No more than one toe per foot may be clipped in birds.
    - Use sharp scissors or a blade sanitized with 70% ethanol or antiseptic solution (Clidox, povidone iodine, chlorhexidine)
    - If bleeding is observed, apply gauze with gentle pressure or styptic powder to the cut digit(s) for hemostasis.

The DLAM veterinary staff is available to discuss alternate identification methods as well as provide toe-clip training. Please contact a veterinarian at x5-2651.

Fluid and Drug Administration

When drugs, vaccines, injectable anesthetics or other agents are to be administered, one or more of several different routes may be selected. The routes selected are governed by the nature of the agent being administered, the animal, the purpose of the administration and other factors. The more common routes of administration used for laboratory animals are classed as follows:

Guidelines for fluid and drug administration or A Good Practice Guide to the Administration of Substance and Removal of Blood, Including Routes and Volumes http://www.aaalac.org/accreditation/RefResources/BloodRemoval.pdf

Gastro-intestinal Tract:

- Oral or per os (PO) - through the mouth
- Gavage - into the stomach via tube

Parenteral:

- Intravenous (IV) - directly in the vascular system through a vein
- Intraperitoneal (IP) - injected into the abdominal cavity
- Subcutaneous (SQ) - injected under the skin
- Intramuscular (IM) - injected into a muscle
- Intradermal (ID) - injected between the layers of the skin

Gastro-intestinal Tract: Substances may be admitted orally by addition to the food or drinking water, by use of a capsule or pill or by instillation into the mouth using a mechanical device, such as a syringe. Capsules or coated pills are rarely used in rabbits or rodents. When used, capsules or pills are placed in the mouth near the back of the tongue, and the animal is induced to swallow by stroking the throat.

Stomach tubes or gastric feeding needles are inserted through the mouth into the stomach or lower esophagus (Figure 7). Care must be taken that the tube does not enter the trachea or the needle puncture the esophagus. In most cases, introduction of the tube toward the rear of the mouth will induce swallowing and the tube readily enters the esophagus. A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea. Flexible or plastic tubes may be bitten or chewed and some care must be taken to prevent this. With rabbits, a dowel of wood or plastic with a hole in the center is inserted behind the incisors. This prevents chewing and permits easy entrance of the stomach tube. Rabbits should be placed in a restraining device before attempting this procedure to avoid unnecessary struggling and injury. A small, curved, metal tube, usually with a ball on the end (feeding needle) is often used with small rodents. Entrance may normally be obtained without anesthesia using ordinary hand restraint and the ball prevents trauma to the esophagus and oral cavity. With the stomach tube fitted to a syringe or aspirator, material may be administered or withdrawn as required. A safe volume to gavage rats and mice is 10 ml gavage solution per kg body weight. DLAM technical staff offers instruction with these techniques.

Figure 7: Rodent Gavage Needle

Parenteral: Parenteral routes of administration involve injections into various compartments of the body. Sites used for collection of blood from veins may also be used for intravenous administration. Intraperitoneal administration is one of the most frequently used parenteral routes, but other commonly used locations are the musculature and the subcutis. Materials given intramuscularly must be small in amounts. Absorption via this route, however, is more rapid than subcutaneous administration. Regardless of the route to be used, it is essential that the subject be securely restrained to avoid injury to personnel, caused by dislodged needles, and to animals because of struggling.

The investigator should know the physiological properties of the substance for injection. Considerable tissue damage and discomfort can be caused by irritating vehicles or drugs. The use of the rabbit foot pad as an injection for antigens, with or without adjuvant, is expressly prohibited since it is a needless and painful procedure. A more suitable site for antigen injection is subcutaneously or intradermally over the dorsal body trunk. In general volumes must be limited to a maximum of 0.1 ml per Intradermal or 0.25 ml per subcutaneous injection site.

The following outline provides basic information on equipment and techniques for parenteral injections in rodents and rabbits. Demonstration/instruction sessions may be arranged with DLAM.

Mouse

Intravenous: Equipment - 27-30g needle, 1 ml syringe, mouse holder, warming lamp. The lateral veins of the tail are the most frequently used veins. Best results are obtained if the tail is immersed in warm water or the mouse is warmed in the cage with a warming lamp. The veins can be seen when the tip of the tail is lifted and rotated slightly in either direction. The tip of the needle can be followed visually as it penetrates the vein. Trial injection soon discloses whether or not the needle is in the vein. Practice and training are essential. This is not an easy technique to master quickly.

Intraperitoneal: Equipment - Syringe and 23-27g 1/2 to 1 inch needle, preferably with a short bevel. The mouse is held as described in Figure 1 and is held in dorsal recumbency in a head-down position. The injection is made in the lateral aspect of the lower left quadrant (Figure 8). The use of a short bevel needle and its insertion through the skin and musculature followed by immediately lifting the needle against the abdominal wall aids in avoiding puncture of the gut lumen. Rapid injection with a large syringe may cause bruising of tissue and hemorrhage from the pressure of the spray and should therefore be avoided. Unless the left leg is immobilized, there is considerable risk of the mouse's movement causing puncture of the viscera. The maximum volume injected IP into a 20 gm mouse should not exceed 2 ml.

Figure 8: Intraperitoneal Injection of the Rat

Intramuscular: Equipment - 26-30 g, 1/2 inch needle with 1 ml syringe. The back and hind leg muscles are the usual sites selected. Due to the small muscle mass available, the volume of drug injected should be limited.

Subcutaneous: Equipment - 25-27 g, 1/2 to 3/4 inch needle and 1 ml syringe. The site usually chosen is the area between the shoulder blades. This route is useful for administration if isotonic replacement fluids (0.9% saline) in the dehydrated animal.

Retro-Orbital Injection of mice:

MATERIALS:
Anesthetic that is described in investigator’s protocol
Insulin syringes with 28 g. needles
Fluid to be injected

PROCEDURE:

1. Anesthetize mouse.

2. Place mouse in lateral recumbency with its feet facing you. If needed, place mouse's head on a stable surface that is slightly elevated from the table top. The medial canthus of the eye should be on the same level as the needle of the syringe. Having the head elevated makes it easier to advance the needle in a straight line rather than at a downward angle as when the head is resting on the table top.
3. Grasp mouse firmly at scruff of neck until eyes bulge slightly. This is the same restraint method as is used for retro-orbital bleeding.
4. Rest your wrist on the tabletop in front of the mouse's head. Hold the syringe between your thumb and 1st or 2nd finger with the bevel of the needle pointing up.
5. Moving your hand in a steady motion, advance needle parallel to the mouse's nose and insert it at the medial canthus into the space between the eye and the surrounding tissue. Insert needle until you feel a tiny "pop" or change in pressure as it punctures the connective tissue surrounding the globe.
6. Inject no more than 50 microliters (0.05 ml) of fluid into the space. If done properly, you will not see any fluid leaking around the eye. If you do, aspirate the fluid without removing the needle, redirect needle slightly, and repeat injection.
7. Remove needle with smooth steady motion.

Rat

Intravenous: Equipment - Depending upon the size of the rat, needles as large as 20 g may be used. One half to one inch length needle is used. A rat holder and warming lamp are also important. The techniques described for the mouse also apply here. Confinement within a cylindrical holder is the usual method for restraint. Light anesthesia with ketamine and Xylazine is helpful for restraint. Prolonged intravenous administration/sampling may be accomplished by jugular vein catheterization. This requires a surgical approach. Please contact DLAM for this service.

Intraperitoneal: Equipment - 23-25 g, 5/8 to 1 inch needle. The location is the same as described for the mouse. Restraint is best accomplished with a second person holding the rat in a head-down, stretched-out position, or with light anesthesia.

Intramuscular: Equipment - 25-26 g, 1/2 to 5/8 inch needle with 1 ml syringe. The back and hind leg muscles are used.

Subcutaneous: Equipment - 23 g, 1 inch needle. The usual site is between the shoulder blades. Be sure and use adequate restraint. Rat skin is thick and difficult to penetrate. Care should be taken to avoid accidental human injections.

Rabbit

Intravenous: Equipment - 22-25 g, needle or butterfly catheter of suitable length with a syringe. A rabbit holder of plastic or metal construction is necessary. The marginal ear vein is used almost exclusively for substance administration (Figure 9). Place the rabbit in a rabbit holder. The hair over the vein is clipped or plucked and the skin cleansed with alcohol. The vein may be distended by flicking with the finger a few times. Holding off the vein near the base of the ear will also help distention. Refer to the next section for blood collection techniques.

Figure 9: Marginal Ear Vein of the Rabbit

Intraperitoneal: Equipment - 20-22 g, 1 to 1 1/2 inch needle with suitable syringe. Smaller needles may be used for small volumes of low viscosity substances. An assistant or chemical restraint is necessary to reduce motion of the limbs. The abdomen is clipped and the skin disinfected. The rabbit is held in a head-down position. Injections are made in the lateral aspect of the lower left quadrants. Caution must be taken to avoid puncturing a distended urinary bladder, the bowel or the liver.

Intramuscular: Equipment - 22-23 g, 1 inch needle. The most frequently used sites are the back muscles lateral to the vertebrae and caudal to the Aab, or the lateral thigh musculature. Volume should not exceed 0.25 ml per site for adjuvant and antigen combinations. If repeated injections are to be made, rotate sites. Adequate restraint is important.

Subcutaneous: Equipment - 20-23 g, 1 inch needle. They are most frequently used is between shoulder blades. Volume should not exceed 0.25 ml per site for adjuvant and antigen combinations.

Intradermal: Equipment - 25-26g, 1 inch needle. The most frequently used site is over the shaved back. Volumes should not exceed 0.05 ml per site for adjuvant and antigen combinations. Injection sites should be spaced 3-4 cm from each other to prevent confluence.

Please contact DLAM staff for information on routes of drug administration in larger laboratory animals such as nonhuman primates, dogs, cats and swine.

Blood Collection

Site Preparation: Certain general procedures and precautions are applicable to methods of blood collection as well as to administration of fluids and anesthetics. When venipuncture is required, hair should be shaved from the site for better visibility. The area of injection should be cleansed with alcohol. Some procedures will require anesthesia; others may be carried out without anesthesia, provided suitable restraint is possible. In order to visualize veins better, one of several methods of dilation may be used. The vessel may be occluded with digital pressure to cause enlargement. Heat will also cause dilation. When using the rabbit ear, or mouse or rat tail, a low watt light bulb may be used for heat. The preferred method of collection of large volumes of blood from the rabbit ear is with the use of a droperidol-fentanyl tranquilizer that promotes arterial dilation and makes blood collection from rabbits simple for even the inexperienced phlebotomist (Drug Dosage Table -Table 1).

Guidelines for blood collection **or** A Good Practice Guide to the Administration of Substance and Removal of Blood, Including Routes and Volumes
http://www.aaalac.org/accreditation/RefResources/BloodRemoval.pdf

Equipment needed: Needles of appropriate gauge and length must be selected with care. For the tail vein or artery of rats and mice, small needles (25-30g) should be used. For other vessels in other animals, the suitable size will depend upon the size of the animal and vessel.

Technique: Proper insertion of the needle into the vein or artery is the most tedious part of the procedure. Certain guidelines may be given, but only practice can be expected to provide any proficiency. A precise, careful introduction of the needle is always best. The needle is inserted parallel above the vessel and the tip directed into the lumen along with the longitudinal axis. The intracardiac puncture generally represents the most practical method of blood collection from small rodents when more than a few drops are required. It is also useful in rabbits for exsanguination. Animals must be anesthetized and placed in dorsal recumbency. The point of the strongest heart beat is determined with the forefinger. The needle is inserted through the skin, between the Aab at this site, directly into the heart. Blood should be withdrawn slowly. The cardiac route for blood collection is a terminal procedure.

In the rabbit, the marginal ear vein is most useful for intravenous injection, but not blood collection. Blood collection is best accomplished from the central ear artery via butterfly catheter or needle (Figures 10 a & b). Thirty to forty ml may be collected in this manner. The absolute maximum of blood to be withdrawn at one times is 9 ml/kg body weight. The PCV (packed cell volume) must be measured at each collection if such large volumes of blood must be withdrawn. If the PCV drops below 35%, collection must be reduced. The use of a droperidol-fentanyl tranquilizer promotes arterial dilation, relaxes the rabbit and makes blood collection from rabbits simple for even the inexperienced phlebotomist (Table 1). DLAM is available to demonstrate or perform this service.

Figure 10: Central Ear Artery of the Rabbit

In the rodent, blood collection by cutting off toes is not permitted. Collection from the tail artery may be increased by warming it in water. Animals should be restrained in restraining device or anesthetized. After cleaning, a small nick is made on the ventral midline of the tail and blood is collected. Digital pressure will stop the blood flow. Withdrawal of blood from the orbital venous plexus of rats and orbital sinus of mice and hamsters is frequently

used. When bleeding the mouse, hamster and rat by the retrobulbar technique, the hematocrit capillary tube is directed toward the major venous structures of the orbit. Knowledge of the location of the venous structures and the technique is essential (Figures 10 & 11). Anesthesia is required for all retrobulbar bleeding procedures. Instruction on all of these blood-collection techniques is available through DLAM.

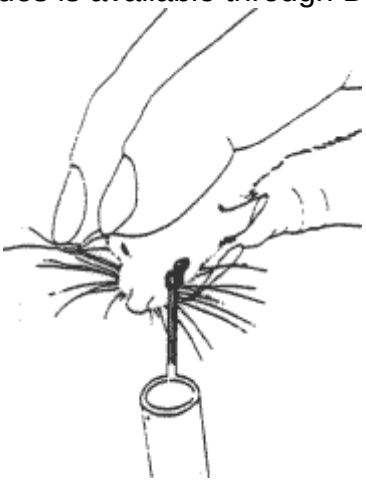


Figure 11: Retrobulbar Blood Sample Collection in the Mouse

Maximum Blood Volume for Survival Collection in Lab Animals

The maximum amount of blood to be collected, as a survival procedure, from the following laboratory animals is 15% of the circulating blood volume. Frequency of collection should not exceed every other week. Hematocrit must be monitored and fluid replacement considered for protocols which require blood collection in larger volumes or at more frequent intervals.

Species	Total Blood Volume	15% Blood Volume
Rabbit	60 ml/kg b.w.	9.0 ml/kg b.w.
Rat	58 ml/kg b.w.	8.7 ml/kg b.w.
Mouse	78 ml/kg b.w.	11.7 ml/kg b.w.
Dog	90 ml/kg b.w.	13.5 ml/kg b.w.
Cat	66 ml/kg b.w.	9.9 ml/kg b.w.

A Good Practice Guide to the Administration of Substance and Removal of Blood, Including Routes and Volumes
http://www.aaalac.org/accreditation/RefResources/BloodRemoval.pdf

Approved Euthanasia Dosage and Techniques References

Manual on the Responsible Care and Use of Laboratory Animals Chapter 2: Biomethodology

1. Harkness J. E., Wagner J. E. The Biology and Medicine of Rabbits and Rodents 2nd Edition, Lea & Febiger, Philadelphia 1983.
2. Wixson S., White W., Hughes H. et. al. "The Effects of Pentobarbital, Fentanyl-Droperidol, Ketamine-Xylazine and Ketamine-Diazepam on Noxious Stimuli Perception in Adult Male Rats", Lab Anim Sci Vol. 37, No. 6, DEC 1987.

3. Wixson S., White W., Hughes H. et. al. "A Comparison of Pentobarbital, Fentanyl-Droperidol, ketamine-Xylazine and Ketamine-Diazepam Anesthesia in Adult Male Rats", Lab Anim Sci Vol. 37, No. 6 DEC 1987.
4. Tillman P., Norman C., "Droperidol-Fentanyl as an Aid to Blood Collection in Rabbits", Lab Anim Sci Vol. 33, No. 2, April 1983.
5. Foster H., Small J., Fox J., The Mouse in Biomedical Research: Volume IV, Experimental Biology and Oncology, Academic Press, New York, 1982.
6. Baker H., Lindsey R., Weisbroth S., The Laboratory Rat: Volume 1, Biology and Diseases, Academic Press, New York, 1979.

Graphics were taken from *AALAS Manual for Assistant Laboratory Animal Technicians* edited by W. Sapanaski and J. Harkness, August, 1984. Text format was drawn from University of Wisconsin's "Technical Information and Training Opportunities for Animal Users" manual.

POLICY ON ASEPTIC RECOVERY SURGERY ON USDA REGULATED NONRODENT SPECIES

The U.S.D.A Animal Welfare Act (9 CFR) requires use of aseptic technique when performing major and minor invasive recovery surgery on USDA-regulated species. Major invasive surgery includes penetration and exposure of the cranium, thorax, abdomen or any procedure producing permanent impairment of physical or physiological functions. Minor invasive surgery does not penetrate a body cavity and includes relatively minor operative procedures such as blood vessel cut down, corneal surgery and eye coil implantation. As required by the U.S. Public Health Service and the University Committee on Animal Resources (UCAR), all vertebrate animal-use protocols, regardless of the funding source, must comply with the guidelines stated in the Guide and the requirements of the USDA Animal Welfare Act.

This policy refers to USDA regulated nonrodent species. If you are working with any rodents covered by USDA regulations such as hamsters, gerbils, mole rats or prairie voles, please refer to the Policy on Aseptic Recovery Surgery on Rodents and Birds.
Investigators who believe that their nonrodent USDA regulated animals require exceptions to the Guide recommendations or USDA requirements should contact UCAR for assistance. Otherwise, investigators using these species are expected to follow this policy.
Click on this link to review the current Policy

POLICY ON ASEPTIC RECOVERY SURGERY ON RODENTS AND BIRDS

The U.S. Public Health Service Guide for the Care and Use of Laboratory Animals states that “In general, unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate site for conducting a surgical procedure (either a dedicated operating room/suite or an area that simply provides separation from other activities), the choice may depend on species, the nature of the procedure (major, minor or emergency), and the potential for physical impairment or postoperative complications, such as infection” (1). As required by the United States Public Health Service (PHS), United States Department of Agriculture (USDA) and the University Committee on Animal Resources (UCAR), all vertebrate animal-use protocols, regardless of the funding source, must comply with the guidelines stated in the Guide and Animal Welfare regulations.
Click on this link to review the current Policy

Chapter 3: Alternatives: Replacement, Refinement, Reduction

An amendment to the USDA Regulations (The Food Security Act of 1985, Subtitle F- Animal Welfare P.L. 99-198) requires investigators to consider alternatives to any procedures likely to cause pain or distress in laboratory animals. In interpreting this requirement, the USDA has mandated that investigators state what databases were used in your literature searches to verify that alternatives have been incorporated where possible. The concept of alternative is interpreted to include the three **R**'s as defined by Russell and Burch:

Reduction, **R**efinement and **R**eplacement*. Investigators always consider alternatives but may be unfamiliar with the concept of the three Rs.

Reduction involves using the appropriate number of animals to answer the scientific question posed and avoidance of unnecessary duplication of studies. In your answer to the question on the U.C.A.R. Protocol Review Form about the rationale for number of animals selected (question # 9, Section A), please state statistical and methodological considerations used to determine the number of animals used. The U.C.A.R. encourages investigators to share tissues from euthanized animals whenever possible. Investigators may submit requests for tissues (e.g. organs, blood) to the attending veterinarian or animal care staff. U.C.A.R. approved protocols that may lend well to tissue sharing at euthanasia will be identified. Conservation of animal species and numbers are encouraged.

Refinement of the protocol involves the use of techniques and procedures to reduce pain and distress. Examples include appropriate use of analgesics and anesthetics, appropriate administration of compounds (e.g. correct volumes and routes) and replacement of procedures with less invasive techniques (e.g. imaging vs. surgery, implanted infusion pumps vs. repeated injections).

Replacement of animals with non animal techniques or with animals lower on the taxonomic scale should also be considered.

In response to this mandate, please indicate the database(s) that you search the literature in your area of research that indicate alternatives have been incorporated where possible. Please state the databases in your answers to question #10b. in Section A of the U.C.A.R. protocol. It is recognized that literature searches may not prove current for cutting edge research. The Investigator's experiences at national and international symposia or communications with colleagues are also important when determining viable alternatives. If you would like more information about how to answer the question of alternatives with respect to your research protocols, please contact the U.C.A.R. secretary (x5-1693).

Websites

- The Alternative Concept
- Animal Welfare Information Center
    - AWIC User Tips
    - Animal Welfare Information Center List of Publications
- The Edward G. Miner Library
  Literature search strategies may be formulated in consultation with the reference librarians at Edward G. Miner Library. The Library offers classes on using the Medline database to assist researchers in performing their own searches. The reference librarian performs literature searches at no charge. Please call Miner Library staff at x5-2487 for more information.
    - Animal Testing Alternatives

* Russel MS, Burch RL, *The Principles of Humane Experimental Techniques,* Special Edition, Universities Federation for Animal Welfare (UFAW), 1992, 8 Hamilton Close, South Mimms, Potters Barr, Here EN6 3QD England.