Oakland University
Office of Research Administration

TRAINING and INFORMATION
MANUAL
ANIMAL CARE and USE

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PREFACE

This manual provides information about the resources and services for using animals in research and education at Oakland University. The manual also is a guide to the laws, standards and policies that impact these activities. Those involved in the use of animals at Oakland University are expected to be knowledgeable about these matters and about the procedures to ensure humane practices and professionally acceptable standards of animal care and use.

PREAMBLE

AAMC Policy on the Use of Animals in Medical Research and Education

The Association of American Medical Colleges (AAMC) strongly affirms the essential and irreplaceable role of research involving live animals in the advancement of biological knowledge, human health and animal welfare. In addition, as animals continue to be vital in segments of the medical education continuum (undergraduate, graduate, and continuing medical education), the AAMC supports this use of animals to meet essential educational objectives.

The AAMC affirms the responsibility of the academic medical community to ensure that the use of animals in laboratory research and medical education is judicious, responsible, and humane, and that the care provided to these animals fully meets accreditation standards and regulatory and legislative requirements. It is the Association’s firm belief that further restrictions on the use of animals in biomedical and behavioral research and education threatens progress in health care and disease prevention.

Therefore, the Association of American Medical Colleges supports the continued availability and humane use of animals in scientific research and the education of physicians. The AAMC strongly condemns violence and the threat of violence against scientists, educators, and institutions that use animals in research and teaching. AAMC member institutions are encouraged to work closely with local, state and federal law officials in order to protect students, residents, faculty, staff, animals and facilities.

The policy statement was approved by the AAMC Executive Council on September 25, 2008.
INTRODUCTION

The Oakland University Training and Information Manual on Animal Care and Use is prepared to assist principal investigators, technical staff, department heads, and all others concerned with the humane care and use of animals in research and instruction. Keeping the document current demonstrates the University’s continued commitment to the humane and proper care and use of animals.

Over the years, many changes have occurred in Oakland University’s Program of Animal Care and Use. Some of the changes reflect the increased sophistication of animal research and the need to use greater precision when planning studies that require the use of animal models. Others changes reflect the University’s strong commitment to the humane treatment and proper use of the animals used in its research and educational programs. Still others reflect acceptance by the scientific community of the need to satisfy public concerns for accountability in animal research. Taken together, the various changes that have occurred in the methods and conditions of animal research have necessitated the publication of this fourth edition of the manual. The manual is considered a “living document” and as such, continues to be updated and revised as required.

Five objectives have guided the preparation of this manual:

1) To provide the reader with an appreciation and basic understanding of the regulatory process and means by which compliance is assured, and the responsibilities that one assumes when choosing to use laboratory animals.

2) To provide a concise, up-to-date accessible source of information about the University’s Program for the Care and Use of Animals and the commitment of the personnel involved with the program.

3) To facilitate communication between and among animal users and the animal care staff in the interest of improving research and responsible animal care and use.

4) To document Oakland University’s commitment to ensuring the humane care and use of animals required in the various research and teaching programs of the University.

5) To document Oakland University’s responsibility for ensuring that all personnel involved with animal care and use are appropriately trained, experienced, and qualified to perform their respective duties.

Some of the information appearing in this manual has been condensed from the primer entitled, “Essentials for Animal Research: A Primer for Research Personnel,” produced in a joint effort by the National Agricultural Library, the USDA, and the University of Illinois at Chicago.

Chapters 2, 3, and 8, of this primer are required reading. The primer can be found online at http://www.nal.usda.gov/awic/pubs/noawicpubs/essentia.htm

Individuals who are uncertain as to the proper procedures to be followed or techniques/skills required to perform their respective duties when working with animals, should contact the manager of the Biomedical Research Support Facility (BRSF) at (248)370-4440.
I. PROCEDURES and PROGRAM for ANIMAL CARE and USE

A. Program Overview

Oakland University’s Program for Animal Care and Use fosters the humane care and use of animals in research and education and adheres to all applicable laws, standards and policies affecting such use. The program applies to all use of vertebrate animals at Oakland University, regardless of the sources of support. The responsibility for implementing the program has been assigned to the Office Research Administration (ORA) subject to periodic review and approval of the implementation procedures by the University’s Institutional Animal Care and Use Committee (IACUC), and the Vice Provost for Research, who serves as “Institutional Official” to the United States Public Health Service (PHS) and the United States Department of Agriculture (USDA).

B. Institutional Animal Care and Use Committee (IACUC)

The IACUC is an important element of Oakland University’s compliance with federal and state regulations and plays an essential role in advising the Institution on all matters related to its program for the care and use of animals. IACUC members are appointed by the president of the University. Members include faculty who are experienced users of animals, at least one non-animal user faculty member and a public member, not affiliated with the University. Permanent members of the committee are the University Veterinarian, the University Surveillance Officer, and the Vice Provost for Research who sits as an ex-officio, non-voting member.

The IACUC conducts the reviews of research and instructional projects for the proper care and use of vertebrate animals that are required by USPHS, USDA, and University procedures. IACUC approval is necessary before investigators and course directors can procure animals or initiate any research, testing, or instructional project involving the use of vertebrate animals. The IACUC concerns itself with a number of aspects of a proposal: the proposed housing and husbandry procedures; the health status of the animals; the provisions for proper veterinary care; the measures taken to minimize the number of animals required to produce valid results and alleviate any potential animal pain or discomfort; and the adequacy of the training and experience of the personnel using and caring for the animals. The IACUC reviews projects for scientific merit only where the issue of merit is deemed to bear on the humane treatment or proper use of the animals. Review for scientific or instructional merit is considered to be the responsibility of the funding agencies or the relevant university unit.

The IACUC also performs program reviews and facilities inspections at least once every six months as required by law and files these reports with the Institutional Official.
C. Responsibilities of the Principal Investigator

Research Investigators and Course Directors are entrusted with an essential role in assuring the humane care and use of animals. In activities they conduct or which are conducted under their direction, they have a direct and continuing responsibility to see that animals are adequately cared for and properly used.

Investigators obtain approval of their projects from the IACUC by completing an Application for Use of Vertebrate Animals in Research, Teaching, or Testing (AUVA). Application forms can be obtained and are managed by accessing and creating an account with the University's Research Application Manager (RAM 3.0) online application system at: https://www2.oakland.edu/research/gcsram/login.cfm

Investigator support is crucial to maintaining high standards of animal care and use in any research or instructional setting. The following recommendations are provided for university faculty and their staff before implementing projects that require the use of animals in their research or teaching.

1. Become knowledgeable about, and conduct all activities involving the care and use of animals in accordance with all regulations, guidelines, and approved policies.

2. Design experiments based on:
   a. Scientific merit
   b. Compliance with animal care and use policies
   c. Meeting goals for animal use of the three R's: Replacement, Reduction and Refinement
   d. Procedures that will minimize animal pain, distress and discomfort

3. Submit clear, complete research protocols (AUVA forms) as required, for approval by the IACUC.

4. Administer research protocols properly by:
   a. Ensuring that all personnel involved are qualified to perform their respective duties properly, effectively, and with regard to their actual involvement with animals.
   b. Maintaining complete records of procedures undertaken during all periods of animal use.
   c. Conducting thorough orientations for all personnel on the rationale for the use of animals in your studies.

5. Maintain a scholarly, sensitive, and respective environment during all animal use situations.

6. Participate in continuing education and training programs designed to keep investigators abreast of the latest regulations, techniques, and procedures in the use of animal research.
7. Emphasize the role of laboratory animals when presenting research results or discussing human diseases with lay audiences and describe the contributions of humanely conducted animal studies to the development of new technologies and treatment capabilities.

II. The Biomedical Research Support Facility (BRSF)

Biomedical Research Support Facility is Oakland University’s core animal research facility. The role of the staff of the BRSF is to offer assistance in the service and regulatory responsibilities, education and training, of all users of the animal facility. Service and regulatory functions include: procurement, housing, husbandry and care of animals; provision of animal health and surgical support services; provision of technical services and consultations in support of research; and monitoring of compliance with regulatory requirements. Educational activities include training university personnel in the proper methods and technical aspects of animal care and use as required or requested. BRSF staff can be contacted by calling (248)370-4440 or (248)370-4441.

A. Animal Procurement Policy and Procedure

ALL animals must be procured through Biomedical Research Support Facility (BRSF) Office, 248-370-4441 or 248-370-4440.

A Notice of Intent to Procure Animal Research Subjects form needs to be filled out in its entirety and submitted with the following information:

- Protocol number
- Purchase order (PO) number
- PI Name
- Department
- Oakland University account number
- Animal species
- Quantity ordering
- Sex of species
- Strain name (this is specific to each vendor and must be complete)
- Weight or age of animals
- Housing Specifications (if any)
- Date of delivery
- Vendor source
**B. Commercial Vendors**

Several of our commonly used vendors may be contacted via the internet for product information, health reports and pricing information.

- Charles River Laboratories [http://www.criver.com](http://www.criver.com)
- Harlan [http://www.harlan.com](http://www.harlan.com)
- Jackson Laboratory [http://www.jax.org](http://www.jax.org)
- Taconic [http://www.taconic.com](http://www.taconic.com)
- Covance [http://www.covance.com](http://www.covance.com)
- University of Michigan - Transgenic Animal Model Core Facility [http://www.med.umich.edu/tamc](http://www.med.umich.edu/tamc) (An excellent web page for links to information regarding transgenic animals)

Deadlines for animal orders are necessary to permit administrative processing of all components of the order, and permit the animal supplier to insure availability of required animals.

**C. Animal Importation/Exportation**

Requests to import/export animals from other institutions requires IACUC approval. The University Veterinarian and the Animal Research Facility Manager from Oakland University must approve the importation of all animals from an exporting institution. The animal health records must be submitted to the University Veterinarian prior to shipment approval. This process may take several weeks. Please contact BRSF Technical Services for assistance at 248-370-4440 or 248-370-4441.

**III. Animal Occupational Health & Safety Program**

The purpose of the University's Animal Occupational Health & Safety Program is to identify risks and hazards specific to contact with laboratory animals and research facilities, and to minimize the health and safety risk of working with vertebrate animals to an acceptable level. The program was developed based on the National Research Council guide, *Occupational Health and Safety in the Care and Use of Research Animals*. Personnel included are those involved in the direct care of vertebrate animals and their living quarters, and those individuals who have direct contact with animals (live or dead), their viable tissues, body fluids or wastes.

**A. The program consists of the following elements:**

The program is based on risk assessment. Participation in the program is required of all animal care personnel and recommended for all other personnel whose activities place them at reasonable risk of injury or illness. Eligible personnel may be identified by principal investigators or course directors at the time they complete their Application for the Use of Vertebrate Animals (AUVA) application form for IACUC review. Individuals may
also participate in the program at their own request. This includes, but is not limited to; all BRSF staff, principal investigators, researchers and their technical staff, instructors involved with animal related work, and other personnel who may reasonably be expected to come in contact with vertebrate animals (some personnel in facilities management, security, or custodial services).

1. A medical history, taken on initial employment by the examining physician;
2. A physical examination;
4. A history of allergic reactions, with referral if deemed necessary, by the examining physician;
5. A chest X-ray if deemed necessary by the examining physician,
6. A tuberculin skin test;
7. A blood chemistry panel
8. Immunization against tetanus and rabies. Tetanus immunization is recommended for all personnel. Rabies immunization is recommended for personnel working with random-source dogs and cats or wild animals.

Any potential hazards associated with the project other than hazards inherent with the use of and exposure to conventional laboratory animals, such as allergies, bites, scratches, zoonoses, etc., must be identified and dealt with appropriately by the principal investigator in their AUVA form. Additional information on zoonoses is provided at the end of this section of the manual.

The responsibility for informing employees about occupational hazards, protection against hazards, and about the relationship between good personal hygiene practices and health, rests with their supervisors. Supervisors must notify employees of any possible exposure in the workplace to hazardous biological, chemical or physical agents and monitor personnel exposure directly when informed that a potential or actual hazard exists. Supervisors must provide protective devices, such as respirators and safety eyeglasses, as required. Employing units must provide noise protection devices and protective clothing (masks, gowns, gloves, shoe covers), when required. Protective clothing is provided routinely to animal care personnel. The use of protective clothing and equipment is recommended for all personnel working with animals.

Standard safety precautions are required to limit or prevent exposure to potentially hazardous agents. These include but are not limited to: radiation exposure badges, personal protective equipment (PPE: gloves, hair bonnets, masks, eye protection, gowns, and shoe covers). For handling potentially infectious animals or associated contaminated material or potentially dangerous chemicals, the facility is equipped with a chemical fume hood, two stationary Class II, Type B2 Biological Safety Cabinets, one portable Class II, Type A2 Biological Safety Cabinet, and one portable AniGARD II Animal Transfer Station. All recommendations of the Radiation Safety and Bio-safety Committees are closely adhered to, such as prophylactic immunizations or protective devices.

All animal protocols involving the use of potentially hazardous agents are assessed by the IACUC and submitted to the appropriate university safety committee for review and
approval prior to receiving full IACUC approval. Representatives from the safety committees are available to the IACUC and BRSF staff for questions, or consultation as potential problems are identified.

The University’s Office of Environmental Health and Safety (EH&S) is responsible for the development, implementation and management of University policies and procedures that are designed to protect employees from occupational illness/injury. Oversight committees (safety committees) charged with identifying and addressing hazards and risks in the workplace include the Bio-safety Committee, Laboratory Safety Committee (addressing fire safety, environmental safety, work related injuries, and all other safety issues not covered by a specific committee), and the Radiation Safety Committee.

Employee health services are provided through the University's on-campus Graham Health Center and Crittenton Hospital's Occupational Medicine Clinic. In addition, EH&S maintains an office at the Biomedical Research Support Facility. BRSF staff has ready access to all institutional policies, including those related to health and safety, via the University's intranet.

**B. Reporting Occupational Injuries and Illnesses**

Non-emergency situations are handled at the University's Graham Health Center (GHC). GHC is located on campus and is open during regular working hours. If an employee is injured (i.e., bitten, scratched, suspected lifting injury, allergy symptoms, needle stick, etc.) or suspects an illness is work related, he/she must report immediately to the BRSF manager, who will fill out an **Authorization to Seek Medical Treatment Form**. In addition, personnel are required to report to the manager suspicious symptoms of unknown origin or serious injuries. The affected employee reports to the Graham Health Center for evaluation, treatment, or referral.

If the injury requires emergency care or occurs during off hours, the manager should arrange for the employee to be transported to Crittenton Occupational Medicine (part of Crittenton Hospital) and complete a **Crittenton Occupational Medicine Program Authorization for Treatment Form**. A copy of the initial report form is kept in the BRSF manager's office.

Work-related injuries and/or illnesses are caused by an event or exposure in the work environment that either causes or contributes to the resulting condition or significantly aggravates a pre-existing injury or illness. Work-related accidents and injuries must be reported to a department supervisor immediately. These injuries must be reported in a timely fashion to ensure that unsafe situations or conditions are addressed immediately and that employees receive the appropriate care and treatment without any delay. In addition, timely reporting of injuries and illnesses ensures compliance with **OSHA 29 CFR Part 1904 Recording and Reporting Occupational Injuries and Illnesses**.
C. **In the Event that an Employee is Injured**

1. Ensure that the employee receives appropriate and prompt medical care and treatment.
   
   a) If an employee is seriously injured, contact the Oakland University Police Department at ext. 3331 or 248-370-3331.

2. Supervisors should complete and sign an **Authorization to Seek Medical Treatment Form** and send it with the employee seeking medical treatment.
   
   a) Employees can be seen at Graham Health Center M-F from 8 a.m. to 5 p.m., contact **Graham Health Center** at ext. 2341 or 248-370-2341.
   
   b) Outside of normal business hours, employees can be seen at **Crittenton Hospital’s Occupational Medicine Department**, contact Crittenton Occupational Medicine at 248-652-5000.
   
   c) After hours services for Occupational Medicine are available through the Crittenton Hospital Emergency Room.

3. Immediately report unsafe situations or conditions to Environmental Health and Safety at 248-370-4196 or Work Control Center 248-370-2381.

4. Supervisors must complete an **Occupational Accident Report** within 24 hours of the reported injury.
   
   a) Completed forms should be forwarded to **University Human Resources c/o Benefits and Compensation Services, 401 Wilson Hall**

All occupational illnesses or injuries are recorded and evaluated by the Office of Environmental Health and Safety. The Office of Environmental Health and Safety documents, investigates, and follows up on all work related injuries.

D. **Zoonotic Diseases – Diseases Transmitted from Animals to Humans**

A zoonotic disease is any disease that may be transmitted from an animal to a human under natural conditions. Zoonotic diseases may pose a risk to OU employees, laboratory personnel, students, and visitors who work with or around animals. Some of these diseases pose a significant health consequence. Most of the animals purchased for OU are specifically raised for biomedical laboratory research. Additionally, since their environment and contact is controlled, the risk for zoonotic disease is low for individuals who work in the laboratory animal facility and have direct contact with laboratory animals. However, occasionally animals come in contact with untrained personnel or other animals in shipment, etc. and thus may become infected with a disease. Finally, investigator's and staff’s own pets present a reservoir for infections that can be transmitted to the laboratory animals.
It is the responsibility of the Principal Investigator (PI) to ensure that personnel are familiar with zoonotic diseases of the animals that they are using in their research or teaching activities. While most animals at OU are free of zoonotic diseases, it is important to be aware of pathogenic organisms that may be carried by animals. Upon request, additional information about specific types of animals and their associated disease conditions may be obtained from the staff of the Biomedical Research Support Facility (BRSF).

Animals primarily used for research or teaching purposes at OU are mice, rats, guinea pigs, rabbits, frogs, and occasionally cats. Allergies to animals are among the most common health problems affecting personnel who care for and use animals in research. Laboratory animal allergies are associated with the inhalation of allergens, such as animal dander and urinary proteins, into the lungs. Although allergies may be associated with any species of animal, in the laboratory most cases are due to contact with mice, rats, and rabbits. Pre-existing allergies to dust mites, pollens and molds, and tobacco smoking are risk factors for the development of laboratory animal allergies. Decreasing allergen contact and exposure time and protective clothing can minimize allergic problems.

1. Transmission of zoonotic diseases can be prevented through a variety of means, including use of protective clothing, prevention of bites and scratches, proper sharps handling procedures, medical surveillance and vaccination programs, and post-injury treatment.

2. Although the following does not include all infections or diseases that are zoonotic, it is a selection to include those that may be of interest in the OU animal laboratories.

a. Lymphocytic Choriomeningitis Virus

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<tr>
<td>1)</td>
<td>Agent – LCM Virus</td>
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<td>2)</td>
<td>Reservoir – Wild mice, murine tumors, hamsters</td>
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<tr>
<td>3)</td>
<td>Signs – Mild influenza-like illness with or w/o CNS signs</td>
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<td>4)</td>
<td>Transmission – Aerosol, direct contact w/infected excretions, skin or mucous membranes, contact w/infected tumors, inhaling dust contaminated w/dried excreta, mouse bites</td>
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<td>5)</td>
<td>Prevention/Control – Control entry of wild rodents into facility, serologic surveillance of rodent colonies, proper sanitation. Decontaminate bite wounds.</td>
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b. Rabies

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<tbody>
<tr>
<td>1)</td>
<td>Agent – Rabies virus</td>
</tr>
<tr>
<td>2)</td>
<td>Reservoir – Mammals, wild animals. Rodents and purpose bred laboratory animals are not likely to be a source of this disease.</td>
</tr>
<tr>
<td>3)</td>
<td>Signs – Fever, headache, change in disposition, paralysis.</td>
</tr>
<tr>
<td>4)</td>
<td>Transmission – Bite wounds, contamination of a wound with saliva, aerosol exposure to the virus.</td>
</tr>
</tbody>
</table>
5) Prevention/Control – Animal vaccination, immunoprophylaxis for humans at risk of exposure.

c. Leptospirosis

1) Agent – Leptospira spp.
2) Reservoir – Cattle, swine, dogs, rats, mice, gerbils, squirrels, hamsters, rabbits, and other mammals.
3) Signs – Fever, headache, leukocytosis, chills, encephalitis, jaundice and hemorrhage.
4) Transmission – Contaminated urine and contaminated water.
5) Prevention/Control – Protective clothing and control of wild rodent entry, use of purpose bred animals, vaccination of animals where appropriate.

d. Cat Scratch Fever

1) Agent – Gram negative bacteria.
2) Reservoir – Cats
3) Signs – Pustule at site of scratch or bite followed by regional lymphadenopathy.
4) Transmission – Cat scratch or bite wound.
5) Prevention/control – Sanitation of wound, restraint of fractious cats.

e. Toxoplamosis

1) Agent – Toxoplasma gondii
2) Reservoir – Cats, raw under cooked meat.
3) Signs – Rare clinical disease. CNS disease, fever. Severe mental retardation in fetus may occur.
4) Transmission – Fecal-oral, handling raw or under cooked meat, transplacental.
5) Prevention/control – Good sanitation, pregnant women should avoid handling cat feces and wash hands after handling raw meat.
IV. REPORT of DEFICIENCIES in ANIMAL CARE and USE

The humane care and use of animals is of paramount importance to Oakland University. The treatment of university-owned animals should be of high quality and in compliance with all federal, state, and local regulations. The law requires that all persons involved or in any way associated with the use of animals in research or teaching know how to report deficiencies in animal care and treatment. Statutory authority for this instruction is found in the 1985 Amendments to the Animal Welfare Act, Title 7, United States Code, Section 2131-2156, PL-99-198. The act requires that "...training for scientists, animal technicians, and other personnel involved with animal care...shall include...methods whereby deficiencies in animal care and treatment should be reported." There are no restrictions on who can report an alleged incident. Individuals who may have specific concerns about animal care and treatment are encouraged to report their observations of deficiencies. Anyone who has knowledge of such a deficiency is obligated to report it to the proper OU officials immediately. Under no circumstances will reporting such incidences in good faith be detrimental to an individual’s standing within the University.

A. Allegations of Animal Mistreatment and Non-Compliance

1) Mistreatment: The wrongful or abusive physical or psychological treatment of an animal.

2) Non-compliance: Not following the established procedures, policies, or protocols. These items may include animal use without approval, deviation from an approved protocol, and facility, management, procedural, or other related deficiencies that reflect on the quality of care and use of animals.

B. Reporting Procedures

Concerns regarding misconduct associated with the care and treatment of animals may be reported to the IACUC Chairperson, any IACUC committee member, the University Veterinarian, the Vice Provost for Research, the Director of Regulatory Support, or the Animal Research Facility Manager. Reports should be as specific as possible, i.e., date, time, species involved, animal/protocol identification number, and the names of University personnel involved. Written reports should be addressed to the IACUC Chair or an individual in the following manner:

IACUC Chairperson or Member (name)
Office of Research Administration
5th Floor Wilson Hall
Oakland University
Rochester, Michigan 48309-4401
PRIVATE and CONFIDENTIAL

Reports of care and use deficiencies may also be given verbally to the IACUC Chair, any IACUC committee member, the University Veterinarian, the Vice Provost for Research, the Director of Regulatory Support, or the Animal Research Facility Manager. A current list of IACUC members may be obtained from the Office of Research Administration at (248)370-2762, or from the IACUC Administrator at (248)370-4440.
Oakland University IACUC's **Report of Deficiencies in Animal Care or Use Form** is available in the Biomedical Research Support Facility (BRSF), or by requesting a form from any member of the BRSF Staff. The form is also available online at, [http://www.oakland.edu/upload/docs/Research/Report%20of%20Deficiencies%20in%20Animal%20Care%20or%20Use%20-%20Updated.pdf](http://www.oakland.edu/upload/docs/Research/Report%20of%20Deficiencies%20in%20Animal%20Care%20or%20Use%20-%20Updated.pdf)

**C. IACUC Response to Deficiencies Report**

The receipt of a written or verbal report of animal care and use deficiency by the IACUC will be immediately brought to the attention of the IACUC Chairperson, and the Vice Provost for Research, who is also the Institutional Official. These individuals will select an additional committee member to constitute a subcommittee to investigate and, where concerns are substantiated, bring about the correction of the reported deficiency. Reports and corrective actions will conform to all applicable university policies. At the next regularly scheduled IACUC meeting, the subcommittee will describe the reported deficiency and the corrective action that has been taken or recommended. If, and when, a letter reporting a deficiency is forwarded to an outside regulatory agency (i.e., OLAW, AAALAC), funding agency (through the Office of Research Administration), or in other instances where notification seems appropriate as determined by the committee, a letter will also be forwarded to the department chair affiliated with the protocol in question. Details of any reports or allegations of deficiencies, findings, or recommendations of the IACUC, as well as administrative or legal actions taken by the committee are considered privileged information and may be released only through official channels, or as required by law.

**D. Protection**

IACUC and subcommittee members will remain honor-bound to respect report confidentiality. Individuals generating a deficiency report shall not be discriminated against or be subject to any reprisal for generating the report. Individuals who desire anonymity may be certain that the IACUC will handle a deficiency report in confidence to the extent permitted by law. Neither administrative action nor retribution of any kind may be taken against a person making a good faith report of deficiencies. This is in accordance with public law [9 CFR, Part 2, Subpart C 2.32 (c) (4)]. According to the **Animal Welfare Act**, "No facility employee, Committee member, or laboratory personnel shall be discriminated against or be subject to any reprisal for reporting violations of the Animal Welfare Act."

**E. Consequences**

Principal investigators are responsible for assuring that all personnel involved in research activities under their direction are aware of the above procedures. Willful mistreatment or abuse of animals may be grounds for suspension of all animal use activities or protocols involved, or other disciplinary actions. Disciplinary action may be appealed through existing procedures.
V. REGULATIONS, REQUIREMENTS, and GUIDELINES for ANIMAL CARE and USE

Since the responsibility for compliance with regulations that affect the care and use of animals ultimately lies with the investigator, it is important he/she have a working knowledge of the basic regulatory requirements. The following are brief descriptions of the primary organizations or regulatory agencies responsible for setting forth and enforcing policies involving animal research.

A. THE GUIDE for the CARE and USE of LABORATORY ANIMALS

Since 1963, the Guide for the Care and Use of Laboratory Animals has served as a primary, contemporary guide for animal care and use in the United States. The purpose of the Guide is to assist institutions in developing and maintaining animal care and use programs that are scientifically, technically, and humanely appropriate and to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles. The Guide is written for a diverse group of users, and its guidelines are intended to be applied to many species of animals in varied settings, types of institutions, and uses. The recommendations are based on published data, scientific principles, expert opinion, and experience with methods and practices that have proved to be consistent with high-quality, humane animal care and use. The Guide often makes general recommendations, the details of which must be addressed locally with specific standard operating procedures, decisions by animal care personnel and users, and institutional animal care and use committee (IACUC) approvals.

The Guide (Eighth Edition 2010) has been updated with regard to regulatory compliance, state-of-the-art techniques and equipment, and consideration of new scientific data. It presents a major transition from engineering to performance standards. This approach necessitates a focus on animal well-being and development or definition of appropriate assessment criteria for specific situations. The Guide encourages continued research into improved methods of animal care and use.

B. ANIMAL WELFARE ACT

The Laboratory Animal Welfare Act was first passed August 24, 1966, as PL-89-544, and authorized the Secretary of Agriculture to promulgate such rules and regulations, and orders as he may deem necessary to effectuate the purposes of this Act.

The original Act set minimum standards of care and housing for dogs, cats, primates, rabbits, hamsters and guinea pigs in the premises of animal dealers and laboratories, and identification of dogs and cats to prevent their theft. It required dealers to be licensed and laboratories to be registered.

In 1970, amending legislation to extend the protection of the Laboratory Animal Welfare Act to all species of warm-blooded animals, pet and exhibition trades as well, was
unanimously passed by both Houses of Congress. The bill was renamed the Animal Welfare Act (AWA).

In 1976, the Secretary of Agriculture also promulgated regulations which specifically excluded rats, mice, birds, horses, and farm animals from the definition of an animal. This exclusionary language effectively excludes over 80 percent of the animals currently used in research, teaching, and testing from coverage under the Animal Welfare Act. These excluded animals are covered under the policies Public Health Service (PHS) and the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC).

In 1985, the Act was further amended with the passage of the Food Security Act of 1985 (PL-99-198) which contained an amendment entitled, "Improved Standards for Laboratory Animals Act." This amendment strengthened the standards for providing laboratory animal care, increased enforcement of the Act, provided for collection and dissemination of information to reduce unintended duplication of experiments using animals, and mandated training for those who handle animals.

The amendment also included development of standards for: the exercise of dogs; provision of a physical environment which promotes the psychological well-being of primates; limitation of multiple survival surgeries; and to require the investigator to consult with a veterinarian in the design of experiments which have the potential for causing pain to insure the proper use of anesthetics, analgesics, and tranquilizers. Each research facility will have to show upon inspection, and include in their annual report, assurances that professionally acceptable standards for the care, treatment, and use of animals are being used during the actual research or experimentation. As part of these standards, investigators are required to demonstrate that they have considered alternatives to any procedures which might cause more than momentary pain or distress in the experimental animals.

The most recent amendment to the AWA (PL101-624) was passed in 1990 and was entitled the Pet Protection Act. The regulations developed to implement this amendment define, the minimum holding period for animals in pounds and shelters, that are sold to dealers, and establishes record keeping requirements for dealers who obtain animals from these sources.

The standards in the Act are absolute minimal standards with an attempt to make them as specific as possible to reduce the need for judgment calls by inspectors. A summary of the rules as they pertain to research institutions is as follows or you can review the AWA in its entirety at: http://www.aphis.usda.gov/animal_welfare/downloads/awr/awr.pdf

A. Oakland University must be registered with USDA

B. The CEO must appoint an Institutional Animal Care and Use Committee (IACUC) to consist of at least three members including a chairman, veterinarian, and one member who is not affiliated with the institution. The IACUC shall:
1. Review animal program, policies, and facilities twice yearly and file written reports of such reviews with the Institutional Official.
2. Review and investigate complaints of noncompliance.
3. Review and approve applications involving vertebrate animals for:
   a. Care and use according to AWA standards
   b. Procedures to minimize pain and discomfort and that the investigator has documented that appropriate alternatives to such procedures do not exist
   c. No unnecessary duplication
   d. Appropriate sedatives, anesthetics, analgesics, veterinarian consulted in planning, no use of paralytics without anesthesia
   e. Animals in chronic pain or distress to be euthanized
   f. Medical care provided by a qualified veterinarian
   g. Personnel on projects are trained and qualified
   h. Appropriate pre and post-operative care
   i. No more than one major surgical procedure unless justified in writing
   j. Methods of euthanasia without evidence of pain or distress
4. Notify the PI, Institutional Official, USDA, and federal funding agency if project is suspended

C. Personnel Qualifications

1. All personnel in animal care and use must be qualified to perform their duties.
2. Training and instruction in animal care and use must be available and provided in:
   - Humane methods of care and use-basic needs, handling, pre- and post-procedure care, aseptic surgery
   - The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress
   - Proper use of anesthetics, analgesics and tranquilizers
   - Methods or reporting deficiencies and violations
   - Utilization of literature search services

D. Veterinary Care - must have a veterinarian trained and experienced in lab animal medicine with the authority to carry out an appropriate program of veterinary care

E. Recordkeeping Requirements - retain for 3 years after completion of such activities:

F. Annual Report - Institutional Official (IO) assures that:

1. Standards are followed
2. Alternatives were considered
3. Location of all animals
4. Common name and number of animals used according to pain and distress categories
G. Police may inspect for missing animals

H. Veterinary inspectors may treat or destroy mistreated animals

I. Must comply with all standards for facilities (according to species requirements), animal health and husbandry standards (according to species requirements), and transportation procedures

C. PUBLIC HEALTH SERVICE (PHS) POLICY

The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals sets forth the requirements that are applicable to all research, research training, biological testing, and related activities involving animals that are supported or conducted by agencies of the PHS. The Office for Protection from Research Risks (OPRR) at the National Institutes of Health (NIH) is responsible for general administration and coordination of the Policy.

The Policy is mandated by the Health Research Extension Act of 1985 (Public law 99-158), and implements the U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research, and Training. Included in the Policy are institutional responsibilities for Animal Welfare Assurances, Institutional Animal Care and Use Committees (IACUC), review of projects, programmatic evaluations, facility inspections, record keeping and reporting. Specific criteria for IACUC review of projects, and frequency and methods of review are described. The information required by the PHS in applications or proposals when animals are to be involved, and PHS responsibilities for implementing the Policy, are also included.

Major Provisions of the Public Health Service Policy:

The Institutional Program must include a list of every branch and major component; the lines of authority for administering the program; the qualifications, authority, and responsibility of the veterinarian(s); the membership of the Institutional Animal Care and Use Committee and the procedures which they follow must be stated. The employee health program must be described for those who have frequent animal contact. A training or instruction program in the humane practices of animal care and use must be available to scientists, animal technicians, and other personnel involved in animal care, treatment, and use. The gross square footage, average daily census, and annual usage of each animal facility must be listed.

The Institutional Status must be stated as either Category One (AAALAC accredited) or Category Two (non-accredited). Institutions in Category Two must establish a reasonable plan with a specific timetable for correcting any departures from the recommendations in the Guide for the Care and Use of Laboratory Animals.
The Office for Protection from Research Risks (OPRR) at the National Institutes of Health, which has responsibility for the general administration and coordination of the Policy on behalf of the PHS, provides specific guidance, instruction, and materials to institutions that must comply with the Policy.  
http://grants.nih.gov/grants/olaw/olaw.htm

D. ADDITIONAL FEDERAL LAWS

Controlled Substances Act (PL 91-513, 1970)

This Act regulates the manufacture, distribution and use of controlled substances. Drugs that have a potential for abuse are classified into one of 5 Schedules, based on their abuse potential. Many of the anesthetic and pain relieving agents that are used in animal research appear on the Schedules. The Drug Enforcement Administration (DEA), an agency of the U.S. Department of Justice, implements the Act. Strict requirements have been established by DEA for record-keeping and accountability. Principal Investigators are responsible for obtaining individual Michigan Controlled Substance License and DEA License if using controlled substances in their research projects. All purchases of controlled substances must be approved by the Laboratory Compliance Manager with the Office of Purchasing and Risk.

Laws Relating to Endangered Species and Wildlife Protection

1. Several federal laws and international compacts are designed to protect animal species that are designated as "endangered." A federal permit must be obtained to import, export, capture, transport, possess or offer for sale any endangered or threatened species. Current listings of protected species and permits for their importation and use can be obtained from the Director, U.S. Fish and Wildlife Service, Department of the Interior, Washington, D.C.

B. The Lacey Act regulates the import, export and interstate shipment of wildlife originating in countries outside the U.S. The importation of harmful species is restricted. Information and permits can be obtained from the Director, U.S. Fish and Wildlife Service. (The Lacey Act was adopted in 1900; regulatory standards are published as CFR 50.10, 50.13, 50.14)

3. U.S. Public Health Service regulations restrict the importation of a number of commonly used laboratory animal species, such as nonhuman primates, dogs, cats, rodents, psittacine birds, and turtles. Information and permits can be obtained from the Director, Quarantine Division, Center for Disease Control, Atlanta, GA 30333.

Good Laboratory Practice (GLP) Regulations; Federal Register (43 FR 59986-60025)

The Food and Drug Administration (FDA) administers the Good Laboratory Practices Act and associated regulations. The regulations apply to non-clinical studies in support of applications for marketing of products that are regulated by the FDA. The regulations
involve extensive record-keeping. GLP standards apply to personnel, animal facilities, animal care practices, equipment maintenance, and study protocols. Generally, the sponsor of a grant or contract is responsible for informing the Principal Investigator that GLP regulations apply. At Oakland University, compliance with GLP regulations is administered through the Office of Research Administration.

E. STATE and LOCAL LAWS


Act 241, of 1947 established a program of registration and inspection of facilities that use and maintain laboratory animals. The goal is to ensure humane care and use of animals. The law requires all Michigan scientific institutions that use animals to register with the state Department of Public Health and to be subject to inspection of their animal facilities by the Department. Since 1978, the law has been applied to all vertebrate animals used in research and, for regulatory purposes, together with Act 368, utilizes the standards in the USPHS Guide for the Care and Use of Laboratory Animals.


Section 2671 affirms that the public health and welfare depend upon the humane use of animals in research, education and testing. Section 2672 specifies the composition of the Animal Research Advisory Board.

The Board's role is to regulate and establish standards controlling the humane use of animals. In effect, Act 241 provides for the registration of animal facilities, Act 368 provides the regulatory standards.

3. Act 224, 1970, Use of Dogs and Cats for Research

Essentially incorporates the standards of the Federal Animal Welfare Act into state regulations. The state law applies only to dogs and cats and inspection responsibility is assigned to the Michigan Department of Agriculture.

4. City and County Ordinances

Several cities and counties in Michigan have adopted ordinances or policies permitting the release of unclaimed and unwanted dogs and cats to approved scientific institutions for use in research and education. Pound (random source) animals that may be acquired by Oakland University are obtained by LAMS through federally licensed dealers or by direct collection, in compliance with the local ordinances and policies and with applicable provisions of the federal Animal Welfare Act and Michigan Act 224.
F. VOLUNTARY STANDARDS

Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International

More than 37 years ago, the Professional Standards Committee of the Animal Care Panel (ACP), now the American Association for Laboratory Animal Science (AALAS), recognized a need for assuring the general public that laboratory animal research was conducted on a professional level and that standard procedures were applied. This concept represents the beginning of the effort to form an accreditation program for laboratory animal care and use.

Incorporated in 1965, as the American Association for Accreditation of Laboratory Animal Care, and now recently renamed the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) has conducted a voluntary accreditation program for laboratory animals for over 35 years.

The mission of AAALAC International is to promote high standards of animal care, use, and well-being and enhance life sciences research and education through the accreditation process. AAALAC International is widely accepted by the scientific community as a demonstration of a high level of responsibility for the use of animals in research, education and testing. AAALAC is not a regulatory body and does not make or enforce regulations. Instead, AAALAC International relies on widely accepted guidelines, such as the Guide for the Care and Use of Laboratory Animals, and other resources. Accreditation is a voluntary process and not required by law. A detailed inspection by members of the AAALAC Council on Accreditation and consultants is conducted triennially to confirm institutional compliance with the standards. There are more than 600 programs accredited by AAALAC International worldwide.

VI. ALTERNATIVE METHODOLOGIES

Essentials for Animal Research: A Primer for Research Personnel
Excerpt: by B. Taylor Bennett, DVM, PhD

In the regulations promulgated to implement the Animal Welfare Act as amended in 1985, the research facility must provide assurances that the principal investigators considered alternative techniques to painful procedures and provide guidance concerning research and testing methods that limit the use of animals or minimize the animals’ distress. In this chapter the reader will be introduced to the classical concept of alternatives with a brief discussion of each major category including a limited number of examples. For more in-depth coverage of the subject, the reader is encouraged to obtain the latest bibliography on alternative techniques available from the Animal Welfare Information Center of the National Agricultural Library (see Chapter 5).
In recent years the term alternative techniques has come into common usage in the current controversy involving the use of animals in research, teaching and testing. It is a term that has different meanings to different people and this difference largely depends on which side of the issue one is found. To many biomedical researchers, alternative techniques refer to those which can be used in addition to the more traditional animal models. These techniques can focus on specific biological functions and in many cases reduce the numbers of animals used. Therefore, these methods are an adjunct to the more commonly used animal models. To the so-called abolitionist who seeks the immediate end to all animal research, teaching and testing, the term alternative refers to those techniques which can entirely replace the use of animals. The dictionary defines alternative as: “offering or expressing a choice.” The dictionary also defines technique as “a method of accomplishing a desired aim.” By combining these definitions, the term alternative technique becomes “one which offers a choice in accomplishing a desired aim.”

In designing an experiment which involves the use of animals to confirm or refute a theory, one should consider all the possible techniques that could be used to gather the necessary data. From this review, choose the method which offers the best chance of generating the necessary information in the most economical manner. Economy, in this context, refers to time, actual cost and the number of animals used. By considering the choices that are available for accomplishing the desired aim of the experiment and choosing the one that offers the best chance for success, one has met the requirements of this literal definition of alternative techniques.

Since a literal definition provides a rather simplistic approach to dealing with our responsibility for reducing the potential pain and suffering of animals that must be used, it is necessary to develop a working definition of the term. In Dr. Rowan’s book, Of Mice, Models & Men, he defines the term alternatives to refer to those techniques or methods that “replace the use of laboratory animals altogether, reduce the number of animals required, or refine an existing procedure or technique so as to minimize the level of stress endured by the animals.” Since stress can be difficult to describe and quantitate, for the purpose of this manual it will be replaced by the term distress. The working definition of alternative techniques thus evolves to “those techniques which replace the actual use of animals, reduce the numbers used, and/or refine the techniques to minimize the potential for the animal to experience pain or distress. This concept of the 3 Rs is not new. It first appeared in a book by Russell and Burch published in 1959 entitled, The Principles of Humane Experimental Technique. In the original work, the authors defined the 3 Rs as follows:

“Replacement means the substitution for conscious living higher animals of insentient material. Reduction means reduction in the number of animals to obtain information of given amount and precision. Refinement means any decrease in the incidence or severity of in-humane procedures applied to those animals which still have to be used.”

In this text the authors included non-recovery techniques in anesthetized animals, as well as tissue culture, as replacement methods. Reduction included statistical techniques which
were designed to reduce the actual numbers needed in the study. The use of better animals was also encouraged as a means of reducing actual numbers used. Refinement referred to techniques that reduced the potential for pain and distress. This approach still holds today. It is the principles of Replacement, Reduction and Refinement that will be covered in this chapter. To attempt to address these issues for all the uses of animals that fall under the general rubric of research, teaching and testing is far beyond the scope of this manual. Therefore, the comments that follow will address only broad issues with some specific examples for the purpose of clarification.

Prior to discussing the replacement of animals with non-animal models, the word **animal** must be defined. On the surface this appears an easy task. Common sense would tell us that an animal is one of the two major kingdoms of living organisms. The dictionary defines an animal as “any of a kingdom of living beings typically differing from plants in capacity for spontaneous movement and rapid motor response to stimulation.” In the Definition of Terms promulgated to implement the amended Animal Welfare Act an animal is defined as:

> "Any live or dead dog, cat, non-human primate, guinea pig, hamster, rabbit, or any other warm blooded animal, which is being used or is intended for use for research, testing, experimentation, or exhibition purposes or as a pet. This term excludes: Birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, and horses and other farm animals such as, but not limited to, livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding management, or production efficiency, or for improving the quality of food and fiber."

The PHS Policy defines an animal as, “any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes.” On the other hand the **Guide** defines an animal as, "any warm blooded vertebrate animal." For the purposes of this manual, and to be consistent with most approaches to discussing alternative techniques, an animal will be any living vertebrate, with the caveat that any model system which moves down the phylogenetic scale from the generally acceptable animal model will be considered an alternative.

Principal Investigators, and IACUC reviewers, are encouraged to use NIH’s guide, “Searching for Alternatives to Painful Procedures Used on Research Animals” when considering research alternatives to painful or distressful procedures on animals. This guide serves as a tip sheet for preparation of the Literature Search for Alternatives that Principal Investigators are required to perform when applying to the IACUC for approval of their research projects. This guide can be accessed at: [http://nihlibrary.ors.nih.gov/training/AlternativesSearchTipSheet5-24-04.pdf](http://nihlibrary.ors.nih.gov/training/AlternativesSearchTipSheet5-24-04.pdf)
A. REPLACEMENT

Alternatives which replace animal models can be classified into the following broad general categories:

- Use of Living Systems
- Use of Nonliving Systems
- Use of Computer Simulation

**In Vitro Techniques** - The most commonly recognized non-animal living systems are those which fall into the broad category of *in vitro* methods such as organ, tissue and cell culture. These techniques afford the investigator the greatest control of the "test subject's" environment. Since these systems will not work when the incorrect combination of atmosphere, humidity, temperature, pH and nutrients are provided, they tend to minimize the effects that non-experimental variables can have on the final outcome of a study.

Generally, when suboptimal environments are provided for an *in vitro* system, the problem becomes one of loss of all experimental results and not just the production of compromised results. The most commonly used of the *in vitro* methods are cell culture techniques for monoclonal antibody production, virus vaccine production, vaccine potency testing, screening for the cytopathic effects of various compounds and studying the function and make up of cell membranes. The potential uses of *in vitro* techniques are almost limitless and will continue to expand as more is learned about the various organs and their component tissues and cells, and as the technology of maintaining in vitro environments improves.

**Invertebrate Animals** - Invertebrates are another type of living system which can be used to replace the more commonly used laboratory animals. Over 90 percent of the animal species thus far identified are invertebrates. An invertebrate which has long been used in biomedical research is the fruit fly, *Drosophila melanogaster* -- a classic model for the study of genetics. This species also can be used for detecting mutagenicity, teratogenicity and reproductive toxicity. The marine invertebrates represent different species which have not been widely investigated. However in neurobiology a number of different marine species have been well characterized and used to study the physiology of the nervous system.

**Micro-Organisms** - The micro-organisms represent a third system which has been used to replace traditional animal models. The Ames mutagenicity/carcinogenicity test uses *Salmonella typhimurium* cultures to screen compounds that formerly required the use of animals. Such systems allow for an almost limitless number of compounds to be tested which can create an interesting dilemma: The more compounds that can undergo screening, the more compounds that will be potentially available to test in animals. Alternative techniques can replace the number of animals at a given step in the screening process. However, use of alternatives may increase the number of compounds that must be finally tested in intact animals.
Plants - Plants offer another alternative living system which can be used to replace animals in studies of basic molecular mechanisms. There is very little morphological and functional difference between the organelles isolated from plants and those isolated from animals. The rigid cell wall of plants, however limits their applicability for use as undisrupted cells.

Use of Nonliving Systems

Chemical Techniques - The most widely used nonliving model system involves the use of modern chemical techniques. This is particularly true of the analytical techniques which can be used to identify substances and to determine their concentration or potency. Immunochemical techniques use the binding capacity of highly specific antibodies to seek out minute quantities of antigen. A classic example of this technique can be demonstrated by the currently used techniques for identifying bacterial toxins. Toxin identification previously required the injection of as many as several hundred mice with supernatant from cultures of suspected contaminating bacteria.

These new antibody techniques save animals and speed up confirmation of a tentative diagnosis. By adding a color marker to the Enzyme Linked Immuno sorbent Assay system (ELISA), the whole process becomes a commercially available test kit such as those used in home pregnancy detection. A test that previously required the use of a rabbit now can be performed using an over-the-counter test kit. There are a variety of chemical techniques that can be used to determine the presence of a particular chemical reaction or the presence of an enzyme necessary for a specific reaction. At the most basic level, the identification of a particular chemical structure in a compound can provide a great deal of insight into the potential reactivity and thus the resulting toxicity of a given substance.

Physical and/or Mechanical Systems - The use of physical and/or mechanical systems to replace living animals of even the highest order has application in teaching specific skills and/or reactions to a well-defined set of predetermined circumstances. The use of computer-linked mannequins in teaching basic principles of medicine and applied techniques can be best illustrated by the mannequins used to train people in cardiopulmonary resuscitation.

Historical data can be used for analysis in a variety of databases commonly used in the field of epidemiology. However, while the body of potentially useful information that already exists in a variety of sources is immense, it may not always be in a format which permits ready accessibility for evaluation. For this reason, retrospective epidemiological studies are often the subject of fairly heated debates. Yet with the increasing access to historical data available on existing computer programs, this problem may to a large extent be overcome in the future.

Use of Computer Simulation
The standout in the alternative techniques controversy is the claim made for computer simulation as a means of virtually replacing the use of living animals. In order for biological phenomena to be adapted to a computer model, the basic processes must be expressed in a mathematical formula. Once a formula is developed then an enormous number of variables can be introduced and swiftly processed. The key element for success is the generation of a program from the mathematical formula. The more complete the formula, the more useful the program. The problem is that many of the questions being asked of an animal model are not defined well enough to develop the necessary mathematical model. As the core knowledge of the biological processes expands so will the opportunities to use computer simulation to replace the number of live animals being used.

B. REDUCTION

In discussing the ways to reduce the numbers of animals used, the definition of an animal and the principle of moving down the phylogenetic scale must also be kept in mind. The four broad categories for reducing the number of animals used are:

1. Animal Sharing
2. Improved Statistical Design
3. Phylogenetic Reduction
4. Better Quality Animals

Animal Sharing

Sharing of animals can significantly reduce the number of animals used within a given institution. Between institutions, sharing is more difficult, but can be effective as demonstrated with the Primate Supply Information Clearinghouse, Regional Primate Research Center (SJ-50) University of Washington, Seattle, WA 98195. This service has reduced the total number of primates used by helping to optimize the usage of those already in facilities throughout the country.

Sharing can be as simple as allowing someone to practice a surgical approach on an animal that has been, or is to be euthanized for other purposes, or providing organs or tissues at the time of necropsy. Sharing becomes more complicated when attempting to maximize the use of control animals, but it can significantly reduce the number used within an institution. If two studies involve the need to perform a sham operation, the administration of compounds by identical routes, the use of standard control diets or the need to condition animals to a particular environment, control animals could be shared within the institution. Animal sharing would require some form of centralized clearing process within the Institutional Animal Care Program to match the needs of the various investigators and their studies.

Improved Statistical Design
Anyone who has ever taken a course in experimental design or applied statistics has been bombarded with the importance of consulting with the statistician during the design phase of the experiment and not when the data collected needs to be analyzed. Improper design of experimental protocols and/or the failure to use appropriate statistical methods can result in the usage of an inappropriate number of experimental animals. A variety of design strategies are available which can reduce the number of animals needed in a given study. Experimental protocols which utilize serial sacrifice, group sequential testing and crossover designs can significantly reduce the numbers of animals required.

The availability of low cost statistical packages for almost every computer on the market permits more and more investigators access to sophisticated data management and analysis. This accessibility makes possible the use of design criteria and complicated statistical analysis which heretofore have been largely confined to institutions with large statistical support units. With this ability at their fingertips, investigators should be able to maximize the analysis of the data generated from each animal used, thus reducing the total numbers of animals necessary for a particular set of data.

**Phylogenetic Reduction**

Projects which can be designed to use one of the myriad of invertebrate species instead of a non-human primate species represent a type of phylogenetic reduction which was discussed as a replacement technique. Such broad jumps across the phylogenetic scale are not always possible, but less dramatic shifts can significantly reduce the numbers of higher species being used in research, teaching and testing. In many instances, the theory of phylogenetic reduction has been blurred by a species’ use as a companion animal with little regard for phylogenetic ranking. The animals chosen for project usage should be the least advanced from a phylogenetic standpoint that will provide the necessary data.

The principle of phylogenetic reduction is generally well accepted as a way to reduce the number of animals used, but it often brings many hidden difficulties. As one descends the phylogenetic scale, the available information on the maintenance and use of these animals in a biomedical setting often becomes difficult, if not impossible, to obtain. When choosing a study model, it is critical that the principal investigator take into account the ability of the institution to provide appropriate care for the species chosen. Phylogenetic reduction is an important means of decreasing the number of animals used, but should be practiced carefully and with the full knowledge of the requirements of the species chosen.

**Better Quality Animals**

It is a rare study in which the initial cost of the animals to be used represents the single most expensive aspect of the study. For this reason it can often be false economy to select the source of the animal based on cost alone. When purchasing laboratory animals, it is important to keep in mind that cost and quality are usually directly correlated. By choosing the best quality animal in terms of health status, the possibility that animals will be lost or data compromised by the intrusion of a concurrent disease condition is minimized, if not
eliminated. Choosing the best quality animals, in terms of genetic status, will virtually insure the consistency of animals from study to study. This requires an institutional commitment to the use of animals of defined health status and limits the investigators to the animal sources approved by the institution. Mixing of animals of different health status is a disaster waiting to happen and may negate all the benefits derived from the use of quality animals.

The role of the investigator and staff in assuring the integrity of an animal colony cannot be overemphasized. In choosing a source of animals, a veterinarian should be consulted to insure that the best animals that can be effectively maintained in the institution are purchased. Animals of different or unknown health status should never share the same environment nor common equipment in the animal facility or in the research laboratory.

C. REFINEMENT

Refinement refers to techniques which reduce the pain and distress to which an animal is subjected. For the purpose of this manual these techniques can be classified into the following broad categories:

Decreased Invasiveness
Improved Instrumentation
Improved Control of Pain
Improved Control of Techniques

Decreased Invasiveness

A hallmark of most of the new diagnostic and therapeutic techniques used in human medicine is the minimal degree of invasiveness that is required to successfully perform a procedure to obtain a given set of data. In many instances these techniques are applicable in the research environment and can be adopted for use in animals. A sophisticated example could be the use of Magnetic Resonance Imaging for results that formerly required euthanasia of multiple animals along a time curve to obtain assay tissue. Today one animal can provide all the information along a given curve. A less dramatic example is the vascular access device which permits repeated samples or injections in a single animal instead of using several animals. Invasiveness reduction methods are available in almost every area of biomedical research, and in project design, it is important to identify and use these methods wherever possible. Not only do they represent an alternative technique, but they generally provide much more consistent and reproducible data.

Improved Instrumentation

**Monitoring Animals** - In this age of microelectronics, fiber optics and laser instrumentation, the potential for refining techniques used in animal experimentation seems almost limitless. Improved instrumentation can minimize animal distress by
reducing the level of restraint and/or manipulation necessary to obtain biological samples. Included in this category are the use of tethers in a variety of species to allow continuous access to the various organ systems, while permitting the animal virtually unrestricted movement within its primary enclosure. The advantages of these systems are numerous, not the least of which is minimizing a variety of nonexperimental variables associated with prolonged restraint.

**Analyzing Samples** - Once obtained, samples can be analyzed in very small volumes for a multitude of parameters. Examples of this can be found in the commercially available diagnostic laboratory equipment which require only microliter blood samples to perform a variety of diagnostic tests. The use of smaller sample sizes permits the use of smaller animal species and prevents the need to euthanatize many of these species to obtain the necessary volume of blood. It is now possible to obtain serial blood samples from small laboratory rodents which reduces the number of animals necessary to obtain data over the length of the study.

**Improved Control of Pain**

The Animal Welfare Act requires "that the principal investigator consider alternatives to any procedure likely to produce pain or distress in an experimental animal" and in any practice which could cause pain to animals that a doctor of veterinary medicine is consulted in the planning of such procedures for the use of tranquilizers, analgesics and anesthetics. Since appropriate anesthetic and analgesic agents can minimize the potential pain and distress experienced by animals, an entire chapter of this manual is devoted to the principles of using these agents. Suffice it to say, that of all the possible ways that the 3 R’s can be utilized this is an area where the laboratory animal veterinarian can often be of most help to the investigator.

**Improved Control of Techniques**

Proficiency in the handling and restraint of animals makes it easier to perform a variety of routine procedures with minimal or no pain or distress to the animals involved. Animals are creatures of habit and when proper handling is part of their regular routine, the degree of distress caused by the procedures is minimized. Animals can be trained or conditioned to accept a variety of procedures which if suddenly forced upon them can be distressful. Almost every animal commonly used in the laboratory responds positively to a little tender loving care. To develop the proper techniques and gain confidence in their use requires training by someone with appropriate experience. This can be the veterinarian, a member of the animal care staff or a fellow investigator. Whomever it may be should be sought out before a new species or technique is incorporated into the study. This will reduce the potential distress of all animals involved in the study up to and including the principal investigator.

**Summary**
The use of alternative techniques has been defined in terms of the present regulatory requirements and the principles of Replacement, Reduction and Refinement were introduced. In summary, the reader should consider a fourth R--Responsibility. The use of animals in teaching and research brings with it a responsibility to minimize animal pain and distress. The adoption of the 3 Rs as part of the process of planning and conducting projects using laboratory animals will go a long way toward implementing Responsibility--the fourth R.

**References**


*Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 86-23.


*Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Revised as of September 1986.


VII. Animal Care and Use: A Nonexperimental Variable

John C. Schofield, BVSc, MRCVS and Marilyn J. Brown, DVM, MS

A. INTRODUCTION

The response of a laboratory animal to an experimental variable is influenced by a variety of genetic and environmental factors. An understanding of these factors is necessary to control their affects and minimize the potential influence of nonexperimental variability on the final outcome of a given experimental protocol. Minimizing nonexperimental variability can optimize the use of animals in a given study.

Since the 1930’s, the concept of genetic makeup, or genotype of an animal, combining with the developmental environment to produce the phenotypic expression of the animal has been well accepted. A useful concept concerning the relationship of genetic and environmental factors—dramatype—was proposed by Russell and Burch in 1959. They defined dramatype to be the pattern of performance in a single physiological response of short duration relative to the animal’s life time. It is determined by phenotype and the immediate environment in which the response is elicited. This concept distinguishes between the developmental environment, which directly interacts with genetic factors, and the proximate or immediate environment, which acts upon the combined system. Simplified, genotype plus developmental environment equals phenotype and phenotype plus the immediate environment equals dramatype. This concept stresses the interrelation of the genetic background of the animal, the environment in which it is raised and housed and the laboratory environment in which the animal is used or tested.

Genotype may be controlled through the use of genetically defined animals produced in structured breeding systems or by genetic engineering. This is easiest to accomplish through the purchase of genetically defined animals from reputable suppliers. In-house breeding programs are difficult and time consuming to maintain in a manner which assures genotypic integrity. If such colonies must be used, it is advisable to consult a geneticist to design a breeding program that produces animals of defined genetic characteristics. A genetic monitoring program might also be required to define the genetic makeup of the animals produced. This can be an expensive proposition and requires some expertise to perform. The phenotype can be influenced by regulating environmental conditions in which the animals are reared. For uniform dramatype, the environmental conditions in which the animals are tested must be controlled.

This chapter will deal with three broad categories of nonexperimental variables: physical factors, chemical factors and microbial factors. Physical factors which will be discussed include: cage design and construction, temperature, humidity, ventilation, light intensity and photoperiodicity, noise, bedding, watering systems, feeding, housing systems, shipping and handling. Chemical factors to be discussed will include contaminants of food, water, bedding, and air. Microbial factors will be discussed in terms of some of the common viral, bacterial and parasitic diseases that can affect laboratory animals. The total of all of the components included in these three broad categories combines with the animal’s genetic background to constitute Russell and Burch’s concept of phenotype and dramatype. It is important to appreciate that our knowledge of the effects of nonexperimental variables is rapidly expanding and the purpose of this chapter is to introduce the reader to this subject rather than present an exhaustive or complete treatise.
B. PHYSICAL FACTORS

The physical environment of laboratory animals may be considered to consist of the animal room, or macroenvironment, and the primary enclosure (cage), or microenvironment. Cage design and composition influence the interaction between micro and macroenvironment. Therefore the temperature, humidity, airflow, concentration of waste gases, illumination and noise levels within the cage may be quite different from that monitored at the room level. Each of these factors represents an important nonexperimental variable that will be discussed in more detail.

Cage Design

Cage design and construction material can influence the study results. Galvanized caging material or rubber bottle stoppers can serve as a source of trace minerals which could affect the results of studies where the level of these compounds is being controlled. Other important considerations include whether contact bedding can be used or if animals must be housed on a wire floor. The type of sample collection may require the use of a metabolic cage, or observation studies may require the use of clear rather than opaque caging. The behavioral characteristics of the animal will also dictate the type of cage design used. For example, some animals require perches, nesting boxes or hiding places, and others require built-in restraint devices such as the squeeze mechanisms often found in primate caging. Reproductive needs may require specific caging features. In some species the male must have a method of escape from an overaggressive female. Many neonates have inadequate homeothermic mechanisms and will become hypothermic if not protected by contact bedding or nesting material placed in the cages.

Temperature and Humidity

The temperature and humidity in the animal room (macroenvironment) should be monitored and maintained within published acceptable limits. The temperature and humidity in the microenvironment is more difficult to monitor and control. Variations in temperature and humidity are influenced by such factors as filter tops, hanging wire or solid bottom caging, population density, animal activity level, cage location, and temperature and humidity in the animal room itself. Variations in temperature and humidity can have a variety of effects. For example, exposure to high temperatures will frequently cause rabbits to lick their fur which can predispose them to the formation of hairballs. Very low humidity has been associated with a rodent lesion called ring tail which is characterized by annular constrictions and can result in loss of the tail. More subtle temperature and humidity effects include: altered drug metabolism, increased disease susceptibility and decreased reproductive efficiency. These examples serve to illustrate the need for controlled temperature and humidity in the animals' micro and macroenvironment and the vital role it plays in the generation of consistent, reliable data.

Ventilation

Ventilation in animal rooms can have significant impact on the health status of the occupants. Excessive odor is often the first indication of a ventilation problem in an animal room; however, the concentration of waste gases at the cage level is usually higher than those detected at the room level. Furthermore, the concentrations capable of causing pathology are much less than human sensory threshold levels. Many design features affect room ventilation including the location, number, and configuration of supply and exhaust ducts. Cage-level ventilation is further affected by the presence and/or type of filter top on the cage as well as the design and location of the cage
relative to the room airflow pattern. Ventilation should be such that it keeps the concentration of waste gases to a minimum, reduces the spread of disease, provides a stable temperature and humidity and avoids drafts.

**Lighting**

Light intensity and photoperiodicity in animal rooms can affect reproductive function and animal vision. The recommendation of the *Guide* for light intensity in animal rooms is 75-125 footcandles (fc). However, prolonged exposure to such levels can cause irreversible retinal degeneration in albino rodents and 25 fc has been suggested as a more appropriate intensity for these species. Variable light intensity control devices such as dimmer switches or multiple bank lighting can be installed to facilitate adequate light for observation and husbandry yet provide lower intensity light for general animal housing. Cage position on a rack can be an important factor and an 80-fold difference in light intensity can exist between the upper and lower shelf locations. Photoperiods or light/dark cycles (usually given in hours as L:D) can modify reproductive behavior and circadian rhythms. A daily light cycle which has 12 to 14 hours of light is usually recommended for most species. It is important to keep the light intensity and periodicity constant. Animal rooms should be equipped with automatic light timers. The presence of windows, either to the outside or to the corridor, can affect reproduction in some animals. Corridor windows may be desirable for observational purposes, but they can provide enough light to affect circadian rhythms in nocturnal animals. As with all environmental factors, the special characteristics of the animal should be taken into consideration when planning light cycles. Duration and type of light can affect estrus behavior. Animals can have their reproductive cycles manipulated by changing the light cycle. This technique has been used in several rodent species, cats, and farm animals. Reversed light cycles can be used to accommodate circadian rhythm, sleep and breeding studies within the normal working hours in an institution. Individual room timers provide a facility with more flexibility to meet a variety of experimental requirements.

**Noise**

Excessive noise can also disrupt animal breeding behavior. Noise at excessive levels can cause mechanical damage to the auditory system in both animals and man. Some effects of noise in animals include audiogenic seizures, eosinophilia, increased serum cholesterol levels and increased adrenal weights. It is recommended that noise levels in animal facilities not exceed 85 decibels (db).

**Caging Accessories**

In addition to the micro-environmental effects of the physical configuration of the primary enclosure as discussed above, other aspects of the cage environment should be considered. The presence or absence of bedding material is dependent on the species and situation. For example, many breeding programs utilize some form of bedding to improve neonatal survival. An ideal bedding material should be dust free, non-palatable, absorbent, and free of microbial and toxic contaminants. The choice of watering system depends on species, experimental design, and management factors. Automatic watering systems are expensive to install but can pay for themselves in labor savings over time. Automatic watering systems should be flushed daily when used with low flow rates, such as in rodent rooms, to avoid stagnation and minimize bacterial buildup. When the study protocol requires delivery of a compound in the water, or measurement of daily intake is needed, water bottles or pans are often used. Choice of feeder and type of food is
also species and situation dependent. Some species such as the hamster are frequently fed on the floor of the cage because their broad muzzle can make obtaining food from some rodent feeders difficult. Some species such as rabbits do not readily tolerate sudden changes in diet composition or formulation. When designing a study, it is important to consult someone knowledgeable in the biology and husbandry requirements of the species to be used, so that wherever possible, species variations are taken into consideration.

**Cage Size - Occupancy Standards**

Consideration should also be given to the cage size. There are specific cage size requirements set forth in the *Guide for the Care and Use of Laboratory Animals* and by the Animal Welfare Act. Cage size requirements depend upon the species, weight or size of the animal(s), number of animals in the cage and breeding status. In addition to the floor space requirements the behavioral characteristics of the species, strain, and sex must be considered when group-housing animals. For very social animals, individual housing may cause stress. Even among social animals, the formation of new groups can result in fatal trauma from fighting. Male mice will often fight when group housed, whereas male rats usually do not. Aggressive behavior can be strain specific; for example, F344 male rats and C57BL mice are generally considered to be more aggressive than other commonly used strains. Even in docile animals, overcrowding can lead to fighting, cannibalism and stress. Breeding activity can be significantly modified by group housing arrangements. For example, group-housing female mice can lead to anestrus with subsequent estrus synchronization with the introduction of a male mouse.

**Shipping**

The effect of shipping animals can be a significant physiological stress. Studies have documented the that prolonged transport, high ambient temperatures, lack of water and the potential for microbial contamination may have on the research data collected from animals exposed to such factors. The provision of climate-controlled transport vehicles and filtered crates decreases these stresses. Even under optimal shipping conditions, it has been shown that it takes 1-5 days for the immune system and body weights to return to normal. It is also important to remember that changes in feed, water, and housing conditions can markedly affect newly arrived animals. Animals should be given an adequate period of time to equilibrate after transport.

**Handling**

The frequency and type of handling an animal receives is another non-experimental variable. Investigators and technicians should be familiar with and skilled in the correct techniques for handling and restraining the species involved. This can prevent injury to either the animal or the handler. Daily husbandry routines may need to be scheduled around the research needs. Close communication between the investigator and the animal care staff can minimize handling stress. For example, collection of biological samples may be performed during routine cage changing. This is particularly useful when chemical restraint is required for either function. Since many animals are creatures of habit, regular handling may reduce stress.

**Chemicals**

Chemicals found in the animal’s environment may be inherently toxic or their metabolism may result in the formation of toxic products. They may directly injure cells or interfere with cellular
homeostasis. The possible effect of a chemical depends on the concentration, the agent’s physiochemical properties, as well as the duration, frequency and route of exposure and potential interactions. These chemicals can influence various body systems. For example, it has been demonstrated that chemicals can affect hepatic microsomal enzymes which have many functions, including the biotransformation of drugs and chemicals and regulation of oxygen radical removal. Such chemical sources include: softwood bedding, room deodorizers, insecticides, and ammonia. Chemicals can also target the immune system. Some insecticides cause lymphopenia. Heavy metals can decrease resistance to disease by the reduction of antibody formation, altered phagocytic capacity of polymorphonuclear cells and macrophages, and suppression of interferon production.

**Food and Water**

Food and water can serve as sources for chemical contamination of research animals. Drinking water may be contaminated with synthetic organic solutes such as pesticides. Trihalomethanes are often found in water supplies as a result of the chlorination process. Some facilities hyperchlorinate or acidify water to decrease microbial contamination; however, these techniques can affect the immune response. Inorganic contaminants may include heavy metals and nitrites. Diets can also be a source of contaminants such as estrogenic compounds, aflatoxins, insecticides, and preservatives. These compounds may occur naturally in plant materials, remain as residues from agricultural use, or be the result of contamination in storage or the processing procedures. Commercial diets assayed prior to shipment are available and the results of this assay are printed on the tag attached to each bag.

**Drugs**

Drug therapy, prior to or during a study, can compromise the data obtained. For example, tetracycline alters the immune cell function through its ability to depress chemotaxis and phagocytosis. Aminoglycosides can have neuromuscular blocking properties, and can have negative inotropic effects on cardiac and arterial muscle. Other agents having neuromuscular depressant activity include tetracycline, lincomycin, and the polymycins. It is important that investigators and the animal care staff communicate about the effect that any medications may have on study animals prior to the initiation of treatment. Similarly, anthelmintics or insecticides given by the animal care staff to treat parasitism problems, could affect research results and must be considered in protocol design.

Anesthetic agents are frequently part of experimental protocols. The researcher should balance appropriate levels of analgesia, anesthesia, and chemical restraint with the possible effects of these agents on the experimental results. For example, the dissociative agent ketamine hydrochloride is widely used in anesthesia and restraint because it is easy to administer, is effective in a wide range of species and has a wide margin of safety. Besides the better known cardiovascular effects of ketamine hydrochloride, this drug also has been shown to affect intracellular cyclic AMP, cellular permeability and calcium channels. A pharmacologic knowledge of these drugs will aid in selecting those best suited for each experimental protocol and allow for more informed interpretation of results. Consultation with the institutional veterinarian regarding the use of anesthetics and analgesics during the planning of potentially painful procedures is now a legal requirement.
Microbial Factors

Pathogenic microbial agents can affect research by causing clinical disease, lesions and death. However, in laboratory animals, infection more frequently is asymptomatic with carriers who develop overt disease when stressed by shipping or experimental manipulation. Animals with latent infection may show no overt disease but research results may be compromised through subtle physiological, biochemical or histological changes.

Bacterial Diseases

Species-specific mycoplasma and bacterial diseases are well documented. There are a number of these pathogens associated with commonly used laboratory animal species. For example, mycoplasmosis is an endemic disease in some conventional rodent colonies. It can cause respiratory and genital tract infections thereby affecting exercise tolerance, sensitivity to anesthetic agents, increased susceptibility to other respiratory pathogens, decreased reproductive efficiency and a variety of immune system anomalies. The investigator using rabbits should be aware of the incidence and significance of pasteurellosis as a cause of acute and chronic disease. Pasteurella multocida is very common in conventional rabbit colonies and can cause upper and lower respiratory tract infections, subcutaneous abscesses, middle and inner ear infection and reproductive tract infections. Some species may serve as asymptomatic carriers of bacterial infections which can cause severe clinical disease in other species; therefore different species should not be mixed. Bordetella bronchiseptica can often be isolated from clinically normal rabbits and rarely causes disease in that species but it can be a significant cause of respiratory disease in guinea pigs. In addition to the species-specific organisms, post-operative infections can be caused by a myriad of bacterial contaminants normally present in the animal’s environment. It is important that invasive surgical procedures be done aseptically to minimize the potential effects of these opportunistic organisms.

Although not experimental variables, there are several bacterial diseases of laboratory animals which can be transmitted to man and therefore are of possible concern to those using animals in research. These may include tuberculosis, salmonellosis, campylobacterosis, and shigellosis. The investigators whose studies involve substantial animal contact should be familiar with institutional guidelines and policies regarding the prevention of zoonotic disease. These should include a program of periodic physical examination, an educational program for personnel, immunization where appropriate and the use of protective clothing.

Viral Diseases

Viral infections in laboratory animals can often be asymptomatic. As with bacterial and mycoplasmal infection, clinical viral disease can occur when an animal is stressed. These viruses can be particularly devastating because the effects on research data may not be recognized, yet still be significant. The effects of these latent viruses have been best defined in rats and mice. Barrier housing of commercially available specific pathogen-free rodents will help eliminate these viruses from a colony. Contaminated tissues, particularly murine tumors, have been implicated in many outbreaks of disease. Tissues should be screened for the presence of contaminants prior to their use in a research facility. It is beyond the scope of this chapter to review all the research implications of viral pathogens currently known; however, a few examples will be briefly mentioned. There are key viral diseases of most common laboratory animals and it is important for
the investigator to work with the institutional veterinarian to become familiar with those viruses and learn how they might affect a particular research project.

Sendai virus, a common viral contaminant in conventional mouse and rat colonies, can cause histopathologic changes in the respiratory tract, immunosuppression, and decreased reproductive efficiency. It can also act synergistically with other respiratory pathogens. A viral disease of mice which is often asymptomatic but serious is Mouse Hepatitis Virus (MHV). This virus has been implicated in wasting syndromes in nude mice. It can cause respiratory, hepatic, and enteric disease. Even in asymptomatic animals, it can cause profound immunological disturbances. Some diseases of laboratory animals are often associated with clinical disease and affect a research study due to high morbidity and mortality rather than the subtle effects of the latent viruses. Canine distemper, feline panleukopenia and measles in macaques are examples of these types of viral infections. Although not as prevalent as bacterial zoonoses, some viruses of laboratory animals can be transmitted to man. Examples of these include; lymphocytic choriomeningitis, Herpes virus simiae and rabies.

**Parasitic Diseases**

Parasites of laboratory animals have also been implicated as nonexperimental variables in research. Some parasites such as *Trichosomoides crassicauda* of rats are capable of causing tumors which could significantly obscure results of a carcinogenicity study. Skin mites of mice have been shown to affect immune parameters. Parasites are also capable of causing significant clinical disease such as the rectal prolapses seen with pinworms in rodents and bowel perforation seen with *Prosthenorchis elegans* in non-human primates. Some parasites of laboratory animals can also be transmitted to man. Examples of these parasites are *Hymenolepis nana* and *Entamoeba histolytica*.

It is important to remember that while laboratory animals may not show clinical signs of microbial infection, the infections can have profound effects on research results. Investigators studying immunological function should be particularly familiar with the potential effects of microbial agents on their research. Transmission of contaminant can occur in tumor or tissue inoculation, from direct transmission or via fomites in the laboratory. Animals of different health status should be strictly isolated from one another and all biologic material should be screened for the presence of viral and other contaminants.

**Summary**

The concepts of Russell and Burch - refinement, replacement, and reduction are generally well accepted in the research community. Adherence to these concepts includes attempting to minimize the nonexperimental variables introduced in this chapter. The maintenance of healthy laboratory animals and the reduction of nonexperimental variables is the responsibility of the animal care facility and the investigator working together in an atmosphere of open communication and cooperation.

**References**


*Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 86-23.


### A. MOUSE – *Mus musculus*

**Normal Physiological Data:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>1.5 years average, 3 years maximum</td>
</tr>
<tr>
<td>Weight, Adult Male</td>
<td>20-40 grams</td>
</tr>
<tr>
<td>Weight, Adult Female</td>
<td>18-35 grams</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>1-1.5 grams</td>
</tr>
<tr>
<td>Breeding Age, Female</td>
<td>50-60 days, 20-30 grams</td>
</tr>
<tr>
<td>Breeding Age, Male</td>
<td>60 days, 20-35 grams</td>
</tr>
<tr>
<td>Estrus Cycle</td>
<td>4-5 days, polyestrous</td>
</tr>
<tr>
<td>Gestation</td>
<td>17-21 days, 19 average</td>
</tr>
<tr>
<td>Weaning age</td>
<td>16-21 days, 10-12 grams</td>
</tr>
<tr>
<td>Begin Dry Food</td>
<td>11 days – Food 1.2 to 1.8g/day Water 1.5 ml/day</td>
</tr>
<tr>
<td>Litter Size</td>
<td>4-12 average</td>
</tr>
<tr>
<td>Time to Re-Breed</td>
<td>Polyestrous - Postpartum Breeding</td>
</tr>
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</tr>
<tr>
<td>Breeding Life, Male</td>
<td>18 months</td>
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<tr>
<td>Mating</td>
<td>Pairs, 1 M-2 F</td>
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<tr>
<td>Chromosome Number</td>
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</tr>
<tr>
<td>Body Temperature</td>
<td>37.5°C, 99.5°F</td>
</tr>
<tr>
<td>Respiration Rate</td>
<td>138 average, 84-230 range</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>470 average, 310-840 range</td>
</tr>
<tr>
<td>Blood Volume % Body Weight</td>
<td>70-80 ml/kg, 6-7%</td>
</tr>
<tr>
<td>Maximum Safe Bleed</td>
<td>7.7 ml/kg</td>
</tr>
<tr>
<td>RBC 1000/CU MM</td>
<td>9.2 average, 7-13</td>
</tr>
<tr>
<td>Hb G/100 ML</td>
<td>11.1 average, 10-14</td>
</tr>
<tr>
<td>PCV ML%</td>
<td>41.8 average, 33-50</td>
</tr>
<tr>
<td>Platelets 1000/CU MM</td>
<td>240 average, 150-400</td>
</tr>
</tbody>
</table>
Sexing

Mice are sexed on the basis of the anogenital distance, which is the distance between the anus and genital papilla. The anogenital distance is greater in the male than the female for all ages – adults, juveniles, and newborns.

Refer to the image below and note that the anogenital distance is large in the male than in the female, as shown in:

1. Neonates (the two animals at the top). Observe the landmarks of the anus and the genital papilla in both sexes. In neonates, the penis and vulva cannot be easily differentiated and so are referred to as a *genital papilla*.
2. Adults (the two animals at the bottom). The genitalia can be differentiated.

Sex Determination in Adults

Note the shorter anogenital distance in the female below. Also, nipples become evident in females at about 10 days of age.
Male mice, like other rodent species, retain an open inguinal canal in adulthood. That is, the descended testicles communicate with the abdominal cavity. Depending on the position in which the mouse is held, the testicles may be retracted into the abdomen or descended into the scrotum. Because of the open inguinal canal, castration of mice requires that the surgeon use caution when applying tension to the testicle. Too much tension can result in the intestines being pulled through the inguinal canal.

**Sex Determination in Juveniles**

Occasionally people experience difficulty in determining gender in mice at weaning age, although the anogenital distances are markedly different between males and females.

Newborns have a subtle difference in the anogenital distance, due to their small size and scale of the anatomical landmarks. In determining the gender of newborns, it’s best to examine several animals side by side to distinguish the males from the females.

The difference becomes more apparent after a few days of age. Another landmark is the presence of nipples in the females from 10 days of age, which are absent in the male. Darker mice are more difficult to differentiate than light colored mice.

**Restraint**

40
Mice are easy to restrain for the purpose of examination, injection and other administrations, and blood collection. It’s important to remember the following:

As a small animal, they can be easily injured if handled roughly. You should learn how to handle them firmly but gently and with confidence to avoid injuring these delicate animals.

Mice are inclined to become aggressive and bite. Although their teeth seldom break through your skin, nevertheless, a bite can hurt! Develop your confidence in handling mice by learning from an experienced mentor and practice hand restraint first on anesthetized mice.

If a mouse does bite you, train yourself not to fling the animal from your hand as a fall can hurt the animal and cause the loss of an animal and possible future genetic contamination of strains and stocks. Instead, remember that the mouse has bitten you in fear and self-defense. At that moment, you and the mouse share a mutual desire to disengage from each other. The best way to remove a mouse that is hanging on to your finger is to train yourself to lower your hand back into its cage. Presented with a retreat to its home cage, the mouse will quickly jump off your finger.
If you are unaccustomed to using syringes and needles and you need to learn how to make injections for the first time, it is helpful to use a small stuffed toy or sock to practice your hand maneuvers for inserting a needle through "skin" and for manipulating the syringe plunger one-handed.

The succeeding screens will show:

1. Common approaches for hand restraint of mice.
2. Two devices commonly used to restrain mice.

**Hand Restraint**

There are two common hand methods for restraining mice, both of which are shown below in videos.

No matter how you will restrain the mouse, mice are picked up the following way:

1. If your mice are in barrier or containment housing, please open their cages in an appropriate hood or laminar-flow workbench.
2. Remove the cage top if they are housed in a filter-top cage.
3. Place the wire lid top sideways on top of the cage.
4. Pick up a mouse by the tail (away from the tail tip) and lift the mouse directly to the wire lid. You will find that the wire lid is a useful area to which the mouse will want to hang on with its front feet, allowing you the opportunity to reposition your grasp.
The two methods of hand restraint shown in the videos differ in how the mouse is grasped. If you will be handling a syringe or another device, you will want to scruff the mouse in your nondominant hand.

- Dorsum Scruff Method:
  In the *dorsum scruff* method, the mouse's back skin is grasped (scruffed) from the occiput to the lumbar area. Your thumb and 2nd, 3rd, and 4th fingers hold the scruff. If you require the head to be fully immobilized, your grasp can include the ears and skin over the top of the head. In this method, the mouse is held by a continuous length of a skin fold.

- Tail Wrap Method:
  In the *tail wrap* method, you transfer the tail from your thumb and forefinger to the 4th and 5th fingers of the same hand. These fingers press into your palm the mouse's tail, close to the animal's rump. Then, your thumb and forefinger grasp a scruff of skin at the occiput of the mouse. In this method, the mouse is held by two points: the occiput and the base of the tail. To further immobilize the hindquarters, you may gently entrap one leg along with the tail beneath your 5th finger. It is important to provide back support to the mouse and avoid hyperextension of its back.

Points to remember:

When picking up a mouse -

1. Pick up the tail at the middle, not the tip. A mouse does not need to be picked up at the base of the tail, like a rat does, because the mouse is light and its weight will not damage its tail.
2. Never dangle a mouse by its tail, but instead lift it directly to the cage wire lid or some other support. When dangled, mice appear anxious as seen by their behavior in hyperextending their feet.
3. Mice feel more secure when given a protective cover, e.g., by an overturned cup-like device. This is a means to hold a mouse securely and comfortably for a few moments.
4. If you need a place to briefly sort and hold your mice, say while you are rapidly administering injections to a cage of mice, each mouse can be placed on the wire lid after its injection. Mice will stay on their wire lid a short while if food blocks are present, due to their instincts for food. If you quickly make all your injections, all the mice can be treated without a mix-up of repeated or skipped administrations.
When scruffing a mouse -

1. Take enough skin to firmly immobilize the animal. Immobilization is important to:
   - prevent the animal from biting you.
   - prevent the animal from being injured if it struggles during a procedure, like an injection.
2. Avoid applying too much tension across the throat area. This could impair the mouse's breathing. Carefully watch the respiratory movements and skin coloration of the mouse. Lessen your grasp of skin if the mouse appears to be struggling to breathe and if areas of the ears, eyes, nose, mouth, and feet turn bluish (cyanotic), which will be more evident in an unpigmented mouse.

Device Restraint

Cylinders and DecapiCones™ are commonly used to restrain awake mice for a procedure. Prolonged restraint (more than the few moments needed for an injection) is known to be highly stressful to mice. Any procedure involving prolonged restraint must be in compliance with your institution's policies, government standards and regulations, and approved by your institution's animal care and use committee (IACUC). This lesson presumes a restraint of short duration for a brief procedure.

Hard (usually plastic) devices are typically cylindrical in shape. Depending on the device design, the mouse is either pulled by the tail through the cylinder or it may be scruffed and directed head first into the cylinder. Many such devices are slotted in the cylinder so that a divider can be positioned for holding the mouse as forward as possible in the device chamber. With such devices, the mouse should be allowed to have enough space lengthwise for its spine to be straight.

Commonly available cylinders have different features:

- Flat-sided cylinder, so the animal is resting on a flat surface and the cylinder is stable on a tabletop.
- Fully rounded cylinder so that the animal is resting on a curved surface and a squared base keeps the cylinder stable on a tabletop.
- Tapered, dark tip in one end of the cylinder so that the mouse can feel hidden and more secure in this part of the device.

Often, a mouse will struggle and hyperventilate while in a cylinder, as though it is in emotional distress. These devices are generally considered humane for conscious mice when used for brief procedures. A common practice is to induce anesthesia by gas, insert the mouse into a restraint device, and then complete the procedure, e.g., a tail vein injection, while the animal is waking up.

A DecapiCone™ is a common restraint device for mice. It is a triangular shaped bag made of heavy plastic, and at the tip there is a small opening so the animal can breathe. Mice generally stay calm and are well restrained in a DecapiCone™.

1. Scruff the mouse and direct its head into the large opening of the DecapiCone™.
2. Advance the animal to the narrow tip, orienting its nose in the small opening.
3. Draw the loose material closed at the animal’s hindquarters.
4. A short piece of "twisty" wire could be tied around the bag, at the base of the mouse’s tail. This would secure the DecapiCone™ around the animal for such procedures as tail vein injection.

**Bleeding**

Small amounts of blood can be obtained by slicing off the tip of the tail. Cardiac puncture or orbital sinus puncture with a micro-hematocrit tube will yield larger volumes. Mice should preferably be anesthetized for these techniques.
**Drug Administration**

**IP Injection**

For an intraperitoneal, or IP, injection, you should first locate the point of entry for the needle.

1. Draw an imaginary line across the abdomen just above the knees (see image above).
2. The needle will be inserted along this line on the animal's right side and close to the midline.
3. As this is a female, you can see that the point of entry is cranial to and slightly medial of the last nipple.
4. Inserting the needle on the mouse's right side avoids the cecum, which is a large fluid-filled organ on the left side of the abdomen. The small intestines (on the right side) are less likely to be punctured by the needle.
5. Inserting the needle too far caudally or laterally from the insertion point shown above would risk making an injection into the rear leg which would injure the muscle tissue.

To perform an IP injection, the mouse must be well restrained so that it cannot move during the procedure. This avoids traumatizing the organs once the needle has entered the abdomen.

1. Insert the needle into the abdomen at about a 30-degree angle.
2. The shaft of the needle should enter to a depth of about half a centimeter.
3. Aspirate to be sure that the needle has not penetrated a blood vessel, the intestines, or the urinary bladder.
4. If no fluid is aspirated, you may inject.
5. Withdraw the needle and return the mouse to its cage.
IV Injection and Blood Collection

Volume Recommendations

The following are volume recommendations (ml) for acute intravenous fluid administration and blood collection in adult mice (average 20 g):

<table>
<thead>
<tr>
<th>Safe injection volume IV</th>
<th>Total blood volume</th>
<th>Safe bleed volume \textsuperscript{a}</th>
<th>Bleed-out volume \textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.0 - 2.4</td>
<td>0.1 - 0.2</td>
<td>0.6 - 1.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Removing greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids to replace the volume of blood collected.

\textsuperscript{b}Animals should be exsanguinated only under anesthesia.

(From Wolfensohn and Lloyd, Handbook of Laboratory Animal Management and Welfare, 2\textsuperscript{nd} Ed., Blackwell Science. 1998.)

Sites and Methods for Blood Collection

Below are the sites that are commonly accessed for blood collection and injection.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Anesthesia</th>
<th>Terminal / Euthanasia</th>
<th>Description</th>
</tr>
</thead>
</table>

47
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Anesthesia Required/Recommended</th>
<th>Terminal Procedure</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Lateral saphenous vein            | Anesthesia not required.        | Not a terminal procedure. | The vein is punctured percutaneously, and blood is passively collected as it pools on the skin. Silicon gel applied on the skin decreases clotting. May be aided by sedation to  
1. Enhance vein visibility by peripheral vasodilation (drug effect).  
2. Reduce animal stress (struggling). |
| Retroorbital puncture             | Anesthesia often required.      | Not a terminal procedure but often a discouraged technique. | Retroorbital puncture must be performed by skilled personnel or the risk of injury to the eye and surrounding structures is high. This method is considered to be painful and may cause blindness.  
This technique is discouraged at many institutions in favor of the lateral saphenous bleeding technique for small volume collections.  
Topical ophthalmic anesthetic may provide pain relief after the procedure. |
| Tail tip amputation               | Anesthesia recommended / may be required. | Not a terminal procedure. | Conducted for genotyping. It is best to anesthetize the mouse when amputating the tail tip. Many institutions have guidelines to allow this procedure without anesthesia prior to weaning and to restrict the length of tail that may be amputated. Please check with your institution for guidelines on this procedure. |
| Cardiac puncture                  | Anesthesia required.            | Generally a terminal procedure. | Many institutions have a policy that cardiac puncture be performed as a terminal procedure, i.e., requiring that the animal be euthanized at the end of blood collection. Check with your institution for guidelines on this technique. |
| Axillary vessels                  | Anesthesia required.            | Terminal procedure; mouse must be euthanized. | Because collecting blood from the axilla is a terminal procedure, the mouse should be deeply anesthetized and then it should be euthanized immediately afterwards before it regains consciousness. |
| Carotid artery                    | Anesthesia required.            | Terminal procedure; mouse must be euthanized. | This method is a terminal procedure, where an incision is made through skin and tissues in the neck area. The animal must be euthanized after blood has been collected and before it regains consciousness. |
Oral Gavage

Oral gavage is the administration of fluids directly into the lower esophagus or stomach using a feeding needle introduced into the mouth and threaded down the esophagus.

Feeding needles have a ball tip that makes them atraumatic on delicate oral and esophageal tissues and reduces the chance of introducing the needle into the larynx. Feeding needles are available in a variety of forms and sizes.

- Reusable needles are entirely made of stainless steel: ball tip, shaft, and hub.
- Single-use needles have a stainless steel shaft, a silicone ball tip, and a plastic hub. The needle shaft can be bent to any desired shape, such as a curve. (A curved needle is easiest and least traumatic for passage down the esophagus.)

Below is a size guide for selection of a feeding needle for use in mice (obtained from Braintree Scientific):

<table>
<thead>
<tr>
<th>Wgt. range in grams</th>
<th>Gauge</th>
<th>Shaft Length</th>
<th>Ball Diameter</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>to 14 gms</td>
<td>24</td>
<td>1&quot;</td>
<td>1 1/4 mm</td>
<td>Straight</td>
</tr>
<tr>
<td>15-20 gms</td>
<td>22</td>
<td>1&quot;, 1 1/2&quot;</td>
<td>1 1/4 mm</td>
<td>Straight</td>
</tr>
<tr>
<td>20-25 gms</td>
<td>20</td>
<td>1&quot;, 1 1/2&quot;, 3&quot;</td>
<td>2 1/4 mm</td>
<td>Straight, Curved</td>
</tr>
<tr>
<td>25-30 gms</td>
<td>18</td>
<td>1&quot;, 1 1/2&quot;, 2&quot;</td>
<td>2 1/4 mm</td>
<td>Straight, Curved</td>
</tr>
<tr>
<td>30-35 gms</td>
<td>18</td>
<td>1&quot;, 1 1/2&quot;, 2&quot;, 3&quot;</td>
<td>2 1/4 mm</td>
<td>Straight, Curved</td>
</tr>
</tbody>
</table>
Procedure for Oral Gavage

Procedure:

1. Select a feeding needle appropriate for the size of mouse. A curved needle is easiest to use and least likely to induce trauma. If using a disposable straight needle, consider bending the needle shaft into a curve.
2. Measure the length of the needle to reach the last rib of the mouse, which indicates the position of the stomach. Note the location of the needle at the animal's incisors which will indicate that the ball tip has reached the stomach. You may wish to mark this location on the needle shaft to know at what point you should stop advancing the needle.

3. Consider anesthetizing the mouse with an inhalant agent in an induction chamber. Otherwise, if awake, the mouse will struggle and there is a risk of trauma to the esophagus. Refer below to the citation about esophageal trauma in rats gavaged awake vs. anesthetized.

4. Scruff the mouse firmly, pulling up the loose skin so that the mouse cannot move its head. Hold the head in vertical alignment with the esophagus.

5. Insert the needle tip behind the incisors and direct it towards the back of the throat. Through the needle, you will feel the hard palate and then the soft palate.

6. An awake mouse will often swallow as the feeding needle approaches the pharynx, facilitating entry of the needle into the esophagus.

During the procedure, monitor the color of the mouse’s mucous membranes. If the mouse becomes cyanotic while you are attempting to insert the needle into the esophagus, you may be obstructing the larynx and blocking respiration.

If the animal struggles or appears to be in respiratory distress, withdraw the needle, allow the mouse to rest a moment, and begin again.

7. Insert the needle down the esophagus. Do NOT force the needle but instead allow the needle to move as though falling by gravity alone. There should be no resistance to the advance of the needle down the esophagus. If you force the needle, you will likely traumatize or even tear the esophagus.

8. Inject slowly.

9. When injection is completed, pull the needle straight out.

10. Although oral gavage should be atraumatic when carried out properly, monitor the mouse after the procedure for any adverse signs.

Consider the use of brief inhalation anesthesia to reduce gavage-associated trauma or death due to injuries of the esophagus. In a study of orally gavaged rats, the authors determined that use of brief inhalation anesthesia:

1. reduced gavage-associated death and euthanasia due to esophageal trauma; and
2. body weight was maintained longer in halothane-anesthetized (vs. awake) rats.

The most commonly observed gross esophageal lesions in non-anesthetized gavaged rats were esophageal impaction, hemorrhage, and/or perforation. However, incomplete vehicle retention during gavage was increased appreciably in halothane-anesthetized animals.
Subcutaneous Injection

Subcutaneous injections are used often to inject anesthetics or to administer fluids for hydration during anesthesia recovery. The subcutaneous route of injection is often abbreviated as SC or SQ. The amount of fluid injected should be limited to volumes that will not overly stretch the skin (which would be uncomfortable) or that will not over-hydrate the animal unnecessarily. Typical volumes injected subcutaneously are in the range of 1 ml or less.

1. Lift the skin over the back to form a tent.
2. Insert the needle at the tent base, being careful to avoid directing the needle at your fingers. Your fingers should be at top of the tent, safely above the point of the needle's entry. Hold the needle parallel to the animal's body to also avoid puncturing underlying structures.
3. Aspirate to ensure that the needle has not entered a blood vessel.
4. Inject the full volume at a moderate rate.
5. Withdraw the needle and then press the skin to seal the needle’s exit hole in the skin and to prevent the fluid from leaking out.
6. Check the animal for any bleeding.
7. Because the fluid has been deposited in the subcutaneous space, you can see and feel the bubble of fluid, called a bleb.

When injecting an awake mouse, place the mouse on the wire lid so it can hang on with its front paws during the injection. Scruff the skin over the back and tent it up. Your hand is both restraining the mouse and presenting the area to be injected. Insert the needle into skin at the base of the tent, aspirate, and then inject.

- Note: Mice will generally not object to a subcutaneous injection when they are allowed to grasp the wire lid.

Anesthetics and Analgesics

The proper use of anesthetics and analgesics in all research animals is both a scientific and ethical imperative. In general, unless the contrary is known or established, it should be assumed that procedures that cause pain in humans also cause pain in animals.

Admittedly, there is no definite data on the degree that rodents experience pain relative to various experimental procedures. However, literature shows that rodents that have received analgesics engage in species-typical behaviors more rapidly compared to control animals following procedures such as laparotomy.

Recognizing Pain

Recognition of pain in mice can be difficult. They are by nature nocturnal, and they often exhibit extremely subtle behavioral changes in response to pain. These changes can frequently only be detected by a trained observer.

The reluctance to move when undisturbed, a hunched still posture, or back arching and twitching, have been associated with painful events. Decreased food and water
consumption resulting in weight loss may also be an indication that the mouse is experiencing pain.

**Analgesic Agents**

**Analgesic agents** should be chosen for their ability to relieve specific types of pain (i.e., local acting agents for superficial skin pain vs. more potent centrally acting agents for visceral pain) as well as the duration of action.

Consideration should be given to administering the analgesic preemptively to prevent the stimulation of pain receptors. This has been shown to reduce the severity and duration of pain following a surgical procedure in humans and other species.

Rodents have been shown to return to a state of normal behavior and food consumption more quickly if given preemptive analgesia.

**Selected Analgesic Agents**

**Opioids** (good pain relief)

- Buprenorphine: 0.05-0.1 mg/kg SQ every 4-12 hours
- Butorphanol: 1-5 mg/kg SQ every 3-4 hours

**NSAIDs (Nonsteroidal anti-inflammatory drugs)** (good pain relief, control inflammation)

- Carprofen, 5-10 mg/kg SQ, every 12-24 hours.
- Meloxicam, 5-10 mg/kg PO or SQ every 12-24 hours.
- Ketoprofen, 1-2 mg/kg SQ, every 12-24 hours.

**Local anesthetic agent** (Injected into tissues to provide localized area of anesthesia in skin and underlying tissues)

- Bupivacaine

**Anesthetics**
Anesthetic choices include **injectable agents** or **inhalation gases** (click on each anesthetic type).

For most experimental paradigms, inhalation anesthesia is a superior choice for mice. Inhalation agents support rapid recovery, and the depth of anesthesia can be more readily controlled.

In general, injectable anesthetics have a much lower margin of safety and are associated with respiratory depression and prolonged recoveries. In addition, because IV access is difficult in mice, injectables are often given in a bolus dose intraperitoneally (IP) or subcutaneously (SC) which doesn’t allow the titration necessary to control anesthetic depth.

Anesthetic choices include **injectable agents** or **inhalation gases** (click on each anesthetic type).

---

**Selected Injectable Agents**

- Ketamine/xylazine: 70-100 mg/kg ketamine + 10-20 mg/kg xylazine, IP
  - Anesthesia 20-30 min
  - Sleep time 60-120 min

- Tribomoethanol (Avertin): 250-500 mg/kg IP
  - Anesthesia 60-240 min
  - Sleep time 15-20 min

- Pentobarbital: 40-70 mg/kg IP
  - Anesthesia 20-40 min
  - Sleep time 120-180 min
Selected Inhalation Agents

- Isoflurane:
  - Induction concentration 3-4% delivered in 100% oxygen
  - Maintenance concentration 1.5-3%
- Sevoflurane:
  - Induction concentration up to 8%
  - Maintenance concentration 3-5%
- Halothane:
  - Induction concentration 3-4% delivered in 100% oxygen
  - Maintenance concentration 1-2%


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Preparing for Surgery

Surgical Patient Preparation
Mice have an extremely high metabolic rate and a high ratio of surface area to body volume. Their high surface area causes them to lose heat rapidly such as when they are anesthetized and placed on a cool surface (like a stainless steel table).

Mice are, therefore, more susceptible than larger species to the dehydration, hypothermia, and hypoglycemia that occur as a result of both anesthesia and invasive surgical procedures.

Rodents don’t have the ability to vomit; therefore, withholding food from mice prior to the procedure is not necessary and, in light of the predisposition to hypoglycemia, not recommended.

**Minimizing the Stress of Anesthesia and Surgery**
Warmed (38°C) lactated Ringer’s solution (1-2 ml SC or IP per 30 gram mouse) given at the time of anesthetic induction will help prevent dehydration and compensate for blood loss.

Heat should be provided with a warm-water circulating pad and/or a warming lamp (with care to prevent overheating and burns). The mouse in the above photo is on a heated hard pad. Wrapping the mouse’s body with bubble wrap or aluminum foil also helps to prevent hypothermia.

Having a well prepared operative arena and a skilled surgeon helps to shorten the length of time the animal is anesthetized and to minimize the stress of anesthesia and surgery on the animal.

**Aseptic Technique**

Aseptic technique is well defined in the *Guide for the Care and Use of Laboratory Animals* and should be used in all species.

Contrary to a commonly held belief, mice are susceptible to subclinical and clinical infections of surgical wounds when aseptic technique has been disregarded. These infections have the potential to alter research studies via effects on the mouse’s immune system.

Applying sterile technique may seem challenging because of the small size of the mouse and the batch nature of operative procedures commonly performed. However, aseptic procedures have been developed that are scaled to the mouse and are practical to perform routinely.
Preparing the Injection Site

Hair should be removed from the operative site, but care must be taken as rodent skin is fragile. Due to the small body size, standard large animal clipper blades are impossible to use.

A size 40 clipper blade or less is suggested. Clippers are marketed specifically for rodent use. The fur can also be plucked, but take care not to rip the skin.

It is important to realize that hair removal and sterile skin preparation contribute to hypothermia. Limit the area clipped and prepped to an area appropriate to the size and location of the incision.

As with larger animals, draping the operative site is recommended and 'rodent-sized' skin drapes are available.

1. Clip or pluck the fur from the incision site.
2. Disinfect the surgical field with povidone solution or chlorhexidine. Because of the small size of the animal, you must avoid drenching its body, which would cause hypothermia. Use either a small sponge or a swab.
   a. Move the sponge in a spiral path beginning at the center and proceeding towards the perimeter.
   b. Discard the sponge when it reaches the edge of the field. This protects the surgical field against contamination brought inward on a sponge.
   c. Use a sterile swab dipped in 70% alcohol to remove the povidone solution, following the same circular motion (moving spirally from the center to the outside of the incision area).
   d. Repeat the application of povidone and rinse with alcohol.
   e. Recommendations differ on how many scrubs (povidone and alcohol) should be done but common practice is 2-3 scrubs. Use a gentle touch while scrubbing so as to avoid abrading the skin, which will cause pain later and may contribute to the development of a post-op infection.
3. Discard your exam gloves and put on sterile surgical gloves.
4. Remove the surgical drape from its outer wrapping using aseptic technique. The inner wrapping is sterile and can be handled by the surgeon wearing sterile gloves. The drape shown in the video is a plastic self-adhesive sheet that clings to the mouse's body. It has a fenestration through which the incision will be made.
   a. Take care not to restrict the animal’s breathing when applying the drape. An adhesive drape stretched taut could press on the animal’s chest and abdomen and restrict respiratory movement.
   b. Using a drape that is larger than the mouse’s body will afford you a large sterile field to work in and will help you maintain asepsis during the surgery.
Sterilization of Instruments

Instruments must be sterilized prior to initial use.

If multiple mice are to be operated on within a single time frame, the instrument tips should be sterilized between mice. This can be easily done through the use of a hot bead sterilizer (as shown below).

If this approach is used, care must be taken not to allow contamination of the instrument handles between animals.

More information on specific surgical techniques and aseptic practices can be found in the [Training in Survival Rodent Surgery CD](http://oacu.od.nih.gov/ppt_slides), distributed free by the Office of Laboratory Animal Welfare (OLAW).

Checking for Perception of Pain

Before beginning the surgery, you MUST ensure that the animal is adequately anesthetized and cannot feel pain. This can be done by gently but firmly pinching the animal’s toe or footpad. You can pinch with your fingers BEFORE you put on your sterile gloves, or use a sterile hemostat or forceps after you have donned sterile gloves. Take
care not to pinch too hard or you could damage the animal's foot or toe. This should be
done periodically to determine that the animal remains anesthetized throughout the
procedure.

Note- remember that anything that touches the foot is no longer sterile. Segregate the
instrument from the other sterile instruments to avoid contamination.

Monitoring Anesthesia

Intraoperative monitoring for depth of anesthesia is as important for mice as it is for larger
animal species.

Anesthetic agents depress respiration, so it is critical to closely monitor the respiratory
rate. Because of the naturally high respiratory rate in mice, apnea even of just a few
seconds duration can be fatal.

Respirations can be observed by watching the movement of the mouse's chest wall.

A fading of pink to a blue color in an albino animal's ears and muzzle indicates critically
inadequate oxygenation that must be immediately corrected.

A more refined method of assessing respiratory function is the use of a pulse oximeter; the
oximeter probe can be placed on the mouse's tongue.

Respiratory arrest can be corrected by chest compression (60 compressions per minute) or
by the administration of doxapram (one drop on the tongue).
Supporting Cardiovascular Function

Cardiovascular function can best be supported by:

- minimizing blood loss during the operative procedure
- providing the mouse adequate oxygenation
- maintaining core body temperature
- providing warmed physiological fluids

For abdominal procedures, lavaging the abdomen with warmed fluids helps to maintain fluid balance and prevent hypothermia.

Body Temperature

Body temperature should be maintained so it does not fall below normal, and therefore it should also be monitored. The use of circulating water or specialized animal heating pads are recommended (electric pads designed for human use are not appropriate).

Body temperature monitoring can be done through the use of a rectal temperature probe designed for use in mice.

Post-Operative Care

Mice should be observed until fully recovered from anesthesia as noted by normal ambulation.

Oxygen should be delivered until respiration rate and depth return to normal.

During the recovery period, the animal should be kept warm and additional fluids given subcutaneously during the first 24 hours following the procedure.

Food supplementation such as fruit or peanut butter is also advised.

Paper bedding material in cages seems to adhere less to fresh incisions than do wood chips. To increase comfort for the animal, different types of bedding should be considered during postoperative recovery.

Analgesia During Recovery

As discussed under “Anesthesia and Analgesia” above, pain relief is an important component of postoperative care.

Observing behavioral signs of pain in mice is difficult, but body weight tracking can help to gauge the animal’s recovery, since mice in pain have decreased food consumption.
The current accepted best practice is to provide analgesia for the first 24 hours following an invasive procedure with additional doses given as warranted due to weight loss or signs of pain.

Minimizing pain for laboratory animals should always be considered. Individual institutional policies and the veterinarian should be consulted for specific analgesic regimens.

**Minimizing Pain and Distress**

**Clinical Signs of Illness**

Achieving humaneness in animal research depends upon the control and, whenever possible, the reduction of animal pain and distress. Minimizing pain and distress also reduces the impact of these extraneous factors on the research, i.e., as sources of non-experimental variation.

For example, in a mouse model of experimental autoimmune encephalomyelitis, implementation of supportive treatment (hydration and nutrition) was shown to protect against loss of body weight and to greatly extend survival of the animals in the study, from 25 to 60 days. (Ref: *Lab Animal*, 29(5): 40-46, 2000.)

**Causes of Pain and Distress in Mice**

Research staff should be familiar with the causes of animal pain and distress. Pain and distress are caused by spontaneous and experimentally-induced disease or injury.

Many factors may contribute to an animal’s distress or discomfort, including extreme homeostatic challenges.

Pain/distress should be minimized to an extent that is possible and compatible with experimental objectives.

Wherever possible, pain/distress should be eliminated.

**Factors in Pain & Distress**

Changes in the following parameters may cause or be associated with animal pain or distress:

- temperature (environmental and body temperature)
- hypoxia
- edema
- blood electrolytes, e.g. hyperkalemia
• dehydration
• environment
  o caging
  o cage mates
  o lighting
  o humidity
  o noise
  o vibration

Note - Smaller mammals experience physiologic changes such as starvation (due to high metabolic rate) and chilling (due to large ratio of body surface area to mass) faster than larger animals.

**Animal Monitoring**

A best approach to reducing non-experimental variation caused by animal pain or distress is to systematically monitor animals after a procedure or when illness is expected.

How often the animals should be monitored depends on:

- the severity of the animal’s condition,
- the expected rate of change in the animal’s status, and
- the impact of the procedure on the animal.

At a minimum, all animals should be evaluated once daily. However, the nature of the procedure and condition of the animal may dictate that the animal be assessed multiple times a day.

As mentioned on the previous screen, smaller mammals may experience physiologic changes such as chilling and starvation faster than larger animals. Therefore, rodents may require more frequent monitoring than larger animals.

Some situations may require hourly or even continuous monitoring during critical periods in which rapid change in the animal’s condition would be anticipated.

**Signs of Pain and Distress**

Signs of pain and distress in rodents are not easy to detect because of their body size, their tendency to conceal outward signs of pain and distress, and their habit of hiding or freezing when disturbed.
Nevertheless, signs of pain or distress can be detected in rodents by carefully observing subtle changes in behavior.

The ability to properly assess pain and distress in rodents requires:

- knowledge of normal rodent behavior and appearance
- a systematic approach to observing clinical signs in rodents

**Clinical Exam**

A clinical exam should include observation of the animals' behavior, appearance, and posture to assess:

- signs of pain or distress
- clinical condition and homeostasis
- measurements of clinical parameters, e.g., body temperature, clinical chemistries

**Assessing Appearance and Behavior**

The first step is a gross inspection of mice for abnormalities in appearance and behavior in their home cage. This assessment takes only a few minutes for the practiced observer.

**From the Cage Exterior**

Routinely inspect the rodents through the top and sides of the cage. Get in the habit of removing the cage from the shelf and looking through all sides of the cage.

Signs of distress may be missed in animals on lower or upper shelves because of low lighting or difficult access.

Baby mice and rats can be inconspicuous within piles of bedding or nestboxes.

Lift the cage wire lid to elicit a response to your presence. This disturbance may prompt the animals to move about the cage.

Examine the animals’ behavior, gait, and hair coat.

Normal mice are inquisitive and explore their cage perimeter.

**Behaviors to Observe**

Abnormal mice may huddle in their cage, or they may fail to move around and explore their cage. In addition, mice may vocalize when approached.
Inspect the animal’s mode and speed of movement. Observe the tail position when the animal moves.

- Is the gait (how it walks) awkward? Observe how all limbs move while walking.
- Does the animal teeter or stumble?
- Is the animal’s back hunched and abdomen tucked while walking?
- Does the animal press its belly to the ground while walking?
- Is the tail held stiff and upright? Or does the tail drag?

Tip: Observe a cage of normal animals for a comparison.

Examine (and treat) an individual mouse by gently restraining the animal. You can move the animal to a separate examination box for detailed clinical inspection.

In recent years, several groups have documented a systematic method to assess mouse facial expression and relate it to pain and distress level. The following published articles describe the system:


**Red Tears**

Stressed mice commonly display red tears or porphyrin staining. Porphyrin is an oily discharge from the Harderian glands located in the orbit behind the eyes.

Porphyrin staining may be seen:

- on the nose,
- around the eyelids,
- or on the medial aspect of the forepaws that become stained through grooming of the face.

Affected rodents may also fail to groom or they may have piloerection of the hair coat (giving a spiky appearance to the hair).
Types of Observations

A common approach to assessing animal appearance and behavior is through observation of the following:

Activity Level
e.g., hypoactivity (hunched, huddled, lethargic), hyperactivity, restlessness, lack of inquisitiveness

Attitude
e.g., arousal, depression, awareness of surroundings

Behavior, Spontaneous
e.g., vocalization, self-trauma, isolation from cage mates. These observations are made without disturbing the animal.

Behavior, Provoked
e.g., vocalization, hiding, aggressiveness, minimal response. These observations are made when the animal is disturbed or even prodded.

Body Condition
e.g., emaciation, missing anatomy

Food and Fluid Intake
e.g., elimination of feces and urine

Fur and Skin
e.g., unkempt or greasy or dull fur; porphyrin staining around eyes and nostrils; cyanotic, pale, or congested mucous membranes or skin (ears, feet, tail); skin lesions; soiled anogenital area

Eyes
e.g., clarity/condition of lens, cornea; position of globe (e.g., sunken in orbit or protruding); condition of eyelids, encrustation

Posture
e.g., hunched back, tucked abdomen; prostrate; head tucked down

Locomotion
e.g., gait, ataxia, lameness, action of each limb, position of tail when ambulating

Neurological
e.g., tremor, convulsion, circling, paralysis, head tilt, coma
**Vital Signs**  
e.g., respiratory distress (open mouth breathing, pronounced chest movement)

**Other clinical parameters that are relevant to your study**  
e.g., presence and status of tumors, infection, or surgical wounds

**Tip:** It is helpful to have blank forms to use as "score sheets" to enter and track each parameter assessed.

**Physical Examination**

After assessing the animals’ appearance and behavior, conduct a physical exam using methods that are appropriate to the species and experimental model.

Performing a clinical exam on rodents is somewhat limited compared to larger animals due to the greater difficulty in venous access and the smaller sampling size of biological fluids. Nevertheless, specific methods and equipment for rodents allow a clinical exam to provide information on animal well-being.

Use quantifiable characteristics whenever possible. These can be tracked over time and compared to a starting baseline or to normal, untreated animals.

Such measurements are not only helpful for clinical assessments, but they can also be useful when compiling research data and writing manuscripts.

You may evaluate:

- Behavior
- Body weight
- Surface lesions (wounds, masses)
- Hydration status
- Body temperature (telemetric methods)
- Blood parameters (Blood collection can be difficult/stressful in mice; may be used to confirm disease or failed treatment.)

**Euthanasia**

The term euthanasia is derived from Greek and means *good death*. Another way of thinking of this is that euthanasia should be a *gentle death*.

Specifically, this means that animals should be killed humanely with minimal pain and distress. Everyone has the responsibility to ensure that if an animal’s life is to be taken, it is done with the highest degree of respect, and with an emphasis on making the death as
painless and distress-free as possible. Euthanasia techniques should result in a rapid loss of consciousness followed by cardiac or respiratory arrest and finally, the loss of brain function. The technique selected should minimize distress and anxiety experienced by the animal prior to its loss of consciousness.

To euthanize a mouse, you must be trained in

- the concepts of euthanasia
- the method to be used
- the proper handling of mice

Methods of euthanasia are classified by the American Veterinary Medical Association (AVMA) as follows. Both the USDA and OLAW endorse these AVMA standards.

1. Acceptable

   The AVMA Guidelines on Euthanasia states that "acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia."

   Such methods are considered humane and are preferred for euthanizing laboratory animals.

2. Conditionally acceptable

   The AVMA Guidelines on Euthanasia states that, "conditionally acceptable methods are those techniques that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature."

   The inclusion of conditionally acceptable methods in your protocol generally require scientific justification before your IACUC will approve their use. Such methods may be humane under controlled circumstances and may be necessary to avoid adverse effects by the euthanasia agent (e.g., an effect on tissues ante mortem) on the research study.

3. Unacceptable

   The AVMA Guidelines on Euthanasia states that, "unacceptable techniques are those methods deemed inhumane under any conditions or that the panel found posed a substantial risk to the human applying the technique."
Unacceptable methods are considered inhumane and are absolutely condemned for use as euthanasia agents. These methods cannot be used on laboratory animals.

In their report, the AVMA Guidelines on Euthanasia also include a discussion of **adjunctive methods** which cannot be used as a sole method of euthanasia but can be used in conjunction with other methods to produce a humane death.

Information on acceptable and conditionally acceptable methods of euthanasia is provided in the pages that follow.

**Acceptable Methods of Euthanasia**

Acceptable methods of euthanasia are listed below. Click on each method to access recommendations for its use from the AVMA Guidelines on Euthanasia. In addition, method descriptions and a video are available for CO₂, which is the most common agent used to euthanize mice.

- Barbiturates
- Inhalant anesthetics
- Carbon dioxide (compressed tanks only) - Neonatal mice euthanized with CO₂ take substantially longer to die than the euthanasia times reported and recommended in the literature for adult mice. Prolonged CO₂ exposure time for neonatal mice can be explained by the mechanisms of action of CO₂ and the innate resistance of the neonate to hypercarbia and hypoxia.
- Carbon monoxide
- Potassium chloride in conjunction with prior general anesthesia
- Microwave irradiation

**Conditionally Acceptable Methods**

Methods that are deemed conditionally acceptable by the AVMA Guidelines on Euthanasia are listed below. Click the link for each method below for recommendations on its use in euthanizing mice.

- Ether
- Nitrogen
- Argon
- Cervical dislocation
- Decapitation

The two physical methods above (cervical dislocation and decapitation) are often used as adjunctive methods to ensure death in mice euthanized with CO₂. The combination of anesthesia by an inhalation agent (CO₂ or an anesthetic gas such as isoflurane) with an adjunctive method is an acceptable procedure for euthanasia.
Diseases

Antibiotic Toxicity  Mites
Bite Wounds  Pinworms/Tapeworms
Chloroform Toxicity  Rectal Prolapse
Chronic Ulcerative Skin Lesions  Respiratory Infections
Ectromelia (mousepox)  Salmonellosis
*Eperythrozoon coccoides* Infection  Lice
Fur-chewing Vices  Tumors
Immunosuppression-induced Early Death  Tyzzer's Disease

References

Federal Laws, Regulations, Policies:

5. *USDA Animal and Plant Health Inspection Animal Care Policy Manual*
   Policy #11 - Painful/Distressful Procedures

Guidelines:


Texts:

B. **RAT – *Rattus norvegicus***

**Normal Physiological Data:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>3 years average, 4 years maximum</td>
</tr>
<tr>
<td>Weight, Adult Male</td>
<td>300-400 grams</td>
</tr>
<tr>
<td>Weight, Adult Female</td>
<td>250-300 grams</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>5-6 grams</td>
</tr>
<tr>
<td>Breeding Age, Female</td>
<td>100 days, 200 grams</td>
</tr>
<tr>
<td>Breeding Age, Male</td>
<td>100 days, 300 grams</td>
</tr>
<tr>
<td>Estrus Cycle</td>
<td>5 days, polyestrous</td>
</tr>
<tr>
<td>Gestation</td>
<td>20-22 days, 21 average</td>
</tr>
<tr>
<td>Weaning age</td>
<td>21 days, 40-50 grams</td>
</tr>
<tr>
<td>Begin Dry Food</td>
<td>12 days</td>
</tr>
<tr>
<td>Litter Size</td>
<td>8-12</td>
</tr>
<tr>
<td>Time to Re-Breed</td>
<td>Polyestrous - Postpartum Breeding</td>
</tr>
<tr>
<td>Breeding Life, Female</td>
<td>1 year</td>
</tr>
<tr>
<td>Breeding Life, Male</td>
<td>1 year</td>
</tr>
<tr>
<td>Mating</td>
<td>Pairs, 1 M-2 F</td>
</tr>
<tr>
<td>Chromosome Number</td>
<td>42</td>
</tr>
<tr>
<td>Rectal Temperature C</td>
<td>37.5</td>
</tr>
<tr>
<td>Respiration Rate</td>
<td>92 average, 80-150</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>350 average, 260-450</td>
</tr>
<tr>
<td>Blood Volume % Body Weight</td>
<td>50-65 ml/kg, 6-7%</td>
</tr>
<tr>
<td>Maximum Safe Bleed</td>
<td>5.5 ml/kg</td>
</tr>
<tr>
<td>RBC 1000/CU MM</td>
<td>8.5 average, 6-10</td>
</tr>
<tr>
<td>Hb G/100 ML</td>
<td>14.2 average, 11-17</td>
</tr>
<tr>
<td>PCV ML%</td>
<td>45.9 average, 40-50</td>
</tr>
<tr>
<td>Platelets 1000/CU MM</td>
<td>330 average, 150-460</td>
</tr>
<tr>
<td>WBC 1000/CU MM</td>
<td>9.8 average, 5-13</td>
</tr>
</tbody>
</table>
Biological Features

Though rats share many anatomical and physiological features with humans, rats have many unique biological characteristics. A knowledge of species-specific characteristics is helpful to effectively manage these animals and to plan experimental procedures for their use. Researchers should be aware of the following practical features of rat anatomy and biology.

Click the following links for a brief description and practical tips. (Click a link again to close the section.)

Anatomy

- Ocular

Rats and mice may develop red staining around the eyes and nostrils when they are distressed, (e.g., by disease, trauma, etc.) or in response to some types of drugs. This staining is due to the accumulation of porphyrins produced by the Harderian gland, a lacrimal gland. Though a normal constituent of tears in rodents, lacrimal porphyrin is produced in limited amounts and rodents keep themselves clean of debris through frequent grooming. Porphyrin staining in distressed animals occurs because stress stimulates porphyrin production in tears, and distressed animals groom themselves less often.

- Teeth

Rats have incisors that are open-rooted, meaning that these teeth grow continuously throughout adult life. A diet of soft foods, i.e., in liquid or powder form, or a developmental jaw malformation will cause tooth overgrowth. Staff must be alert to detect any signs of this condition and to provide appropriate treatment. Teeth can be trimmed with small, sharp scissors or nail-trimmer type cutters. Ask your veterinary staff to help or to perform the task for you.
The photo shows a rat with overgrowth of the incisors (upper and lower) and jaw malalignment.

Gastrointestinal

- Inability to vomit

Rats do not vomit because they lack the neurophysiological mechanisms for emesis. Therefore, presurgical fasting is not necessary in rats, as it is for nonrodent species.

- Gallbladder

Unlike mice, rats do not have a gallbladder. Bile passes from the liver through a bile duct directly to the duodenum.

- Coprophagy

In rats, herbaceous foodstuffs are broken down by microbial action in the cecum, which is a large organ in the rat. To assimilate the microbial byproducts of digestion, the rat regularly eats its own feces, a habit known as coprophagy. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the rat.

Metabolism

- Albinism

Most rats used in research are albinos, such as the Sprague Dawley stock. Albinism in rats is an inherited disorder of melanin metabolism caused by the lack of the enzyme tyrosinase, which has an impact both on melanocytes and neurons. Neuronal
morphological abnormalities and functional impairments involve the following sites: medial vestibular nucleus, cochlear nuclei, and retina. Studies comparing albino and pigmented animals have shown differences even in pharmacotoxic kinetics in these tissue areas.

- High rate of metabolism – impact on drug clearance

The rat’s high rate of metabolism produces a rapid clearance of drugs from the body. Drugs administered at dose rates used in larger species (with lower metabolic rates) would reach lower blood concentrations and exert less pharmacological effect in the rat. As a result, rats should receive drug doses that have been scaled to the rat’s metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

In general, rat-specific dose rates have been determined and are widely published for drugs commonly used in animal research, such as anesthetics, analgesics, sedatives, and antibiotics. Investigators are advised to obtain rat dose rates from laboratory animal references or from their institution’s veterinary staff.

- High surface area – impact on hypothermia

Rats have a large body surface area (relative to body volume) plus many hairless body parts (tail, ears, feet). As a result, rats are vulnerable to a profound hypothermia under conditions of sedation and anesthesia. Sedation and anesthesia induce hypothermia due to drug effects on the hypothalamus and a rapid drop in core body temperature. If surgery is being performed, additional heat is lost by convection from an open incision during surgery.

Rats should have a source of warmth throughout a procedure that lowers their body temperature (e.g., anesthesia, surgery) and afterwards until they recover the ability to thermoregulate themselves.

Assessing Pain and Distress

If your proposed study involves a painful procedure, the animal use protocol application may ask for a method of assessing if the rats are experiencing pain or distress.

Assessing pain and distress in rats is difficult at times. This is because rats, like many other species, commonly conceal outward signs of moderate pain and distress. In this case, the behavioral changes that reveal a rat’s pain and distress may be subtle and detectable only with specialized behavioral methods.
Clinical Signs

Severe pain and distress causes overt clinical signs in rats. Laboratory staff working with rats should be trained to recognize abnormalities in:

- Activity level
  - Hypoactivity
  - Hyperactivity
  - Lethargy
  - Restlessness
- Behavior
  - Vocalization
  - Self-trauma
  - Isolation from cage mates
  - Aggressiveness
  - Ataxia
- Appearance
  - Unkempt greasy fur
  - Porphyrin staining around eyes and nostrils
  - Hunched posture
  - Cyanosis
  - Pale mucous membranes
  - Soiled anogenital area
- Vital signs
  - Respiratory distress
- Body condition
  - Weight loss
- Emaciation
- Dehydration
- Intake
  - Reduced intake of food and water

The image below shows porphyrin staining and encrustation on a rat's nose. This is a nonspecific sign of pain or distress.

Chronic Pain or Distress

A chronic state of pain or distress may be more subtle and difficult to detect. A good knowledge of the animal’s normal appearance and behavior is especially important to recognize chronic pain or distress.

Procedures for Injections and Blood Collection

Volume Recommendations

The following are volume recommendations for intravenous fluid administration and blood collection in adult rats (250-500 g):

<table>
<thead>
<tr>
<th>Intravenous Volume max. acute admin.</th>
<th>Total Blood Volume</th>
<th>Safe Bleed Volume</th>
<th>Bleed-out Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 1.0 ml</td>
<td>64 ml/kg</td>
<td>5.5 ml/kg</td>
<td>7.5 - 15 ml</td>
</tr>
</tbody>
</table>

aRemoving greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids
to replace the volume of blood collected. In addition, monitor the animal’s hematocrit for anemia.

bAnimals should be exsanguinated only under anesthesia.

From:


**Peripheral Vessels**

Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 23 to 27 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail vein</td>
<td>1. Accessing the tail vein and the lateral saphenous vein:&lt;br&gt;  o Does not require anesthesia.&lt;br&gt;  o May be aided by sedation because vein visibility is enhanced by peripheral vasodilation (drug effect).&lt;br&gt;  o May be aided by sedation to reduce animal struggling due to distress.&lt;br&gt;  2. Blood collection from the lateral saphenous vein does not involve cannulation of the vein lumen. Instead, the vein is punctured percutaneously and blood is passively collected as it pools on the skin.</td>
</tr>
<tr>
<td>Lateral saphenous vein</td>
<td></td>
</tr>
<tr>
<td>Jugular vein</td>
<td>Commonly performed under anesthesia because of restraint method and the need for animal immobilization.</td>
</tr>
<tr>
<td>Penile vein (males only)</td>
<td></td>
</tr>
<tr>
<td>Cardiac puncture</td>
<td>1. These two methods require anesthesia.  2. Cardiac puncture is most often allowed only as a terminal procedure.  3. Check with your institution for guidelines on this route of blood collection.</td>
</tr>
<tr>
<td>Carotid artery</td>
<td></td>
</tr>
</tbody>
</table>
1. Retroorbital puncture is controversial because of the risk of injury to the optic nerve and other nearby structures.
2. This method is considered to be painful and may cause blindness.
4. Topical ophthalmic anesthetic is recommended post-procedure.

**Nonvascular Injections**

Below are the nonvascular routes of injection that are commonly used in rats. Included are volume recommendations for the safe administration of fluids acutely in adults (average 200 g). Recommended needle sizes are 23 to 27 gauge; larger needles may be necessary for injecting viscous materials.

Subcutaneous (SQ or SC) - 25 ml/kg
Intraperitoneal (IP) - 25 ml/kg
Oral (PO) - 10 ml
Intradermal (ID) - 0.05 ml/site
Intramuscular (IM) - 0.1 ml per site

From:

**Analgesics, Sedatives, and Anesthetics**

Because rats have a high rate of metabolism, drugs are rapidly eliminated from their bodies. Dose rates appropriate for larger species produce ineffective drug concentrations in the bloodstream when used in rats.

This lesson includes dose rates for common drugs and drug regimens used in rats. If you need to use other drug agents, check with your institution’s veterinary staff for assistance in determining a dose rate appropriate for use in rats.
Drug Types

Click the links for the drug types below for doses of common agents and drug regimens that may be used in rats:

- **Analgesics:**
  Available in two drug types – the opioids and the nonsteroidal anti-inflammatory drugs (NSAIDs). The rapid clearance of many of these drugs in rats results in the need for an increased frequency of administration.

- **Sedatives:**
  Sedatives are used to calm animals and facilitate handling, but have no effect on consciousness, the perception of pain or other sensations. When combined with general anesthetics, the result is a balanced anesthesia where muscle relaxation, unconsciousness, and analgesia are enhanced.

- **Sedatives + Analgesia:**
  Some sedatives also have analgesic effects. When combined with general anesthetics, a balanced anesthesia is attained, and these sedatives enhance analgesia through specific effects.

- **Anesthetics:**
  Because rats metabolize drugs so rapidly, many anesthetic agents have brief durations of effect. An anesthetic regimen should be chosen to match the duration of drug effects with the length of the procedure. In particular, short-acting agents (and regimens) should be not be used for long procedures because repeat drug administrations, necessary to prolong anesthesia, will produce uneven blood concentrations and therefore an uneven plane of anesthesia. For long procedures, gaseous anesthesia is often the most practical method to sustain uniformly adequate levels of anesthesia.
Hypothermia

The practice of using hypothermia as an anesthetic for neonates is not recommended. It is not clear whether the depression of neural function by hypothermia is sufficient to prevent the sensation of pain related to a surgical procedure. Also, the recovery from hypothermia may be a painful experience in animals, as it is known to be in humans.

Surgery

Aseptic technique should be used when performing survival surgery on rats. The standards described here are consistent with the Guide for the Care and Use of Laboratory Animals.

If non-survival surgery will be performed (when the animal is euthanized before regaining consciousness), gloves should be worn by the surgeon, the area and instruments should be clean, and the surgery site should be clipped.

Location

Surgery on rats should be performed in a location that allows for a physical separation of the operative field from other functions of the procedure (such as animal preparation and anesthetic recovery) and other activities. The space should be dedicated to surgery during
that time. An animal procedure room within the animal facility is an ideal location, but other locations may be permitted according to institutional policy.

- The isolation of the operative field avoids contaminating sterile areas with animal fur, bedding, nonsterile supplies, etc.
- The location used for the operative field should be cleaned and sanitized before use.
- Materials and supplies used in support of the procedure should be positioned and managed to avoid contaminating sterile areas.

If using shared space during the surgery (such as a procedure room) placing a "do not disturb" sign on the entrance door during the procedure will help minimize contamination.

Aseptic Technique

Survival surgical procedures in rats should be conducted using aseptic technique. Nonaseptic methods are not acceptable. Aseptic surgery includes:

- Preparation of the surgeon
  - Head cover, gown, face mask and sterile gloves should be worn
- Preparation of the patient
  - Clipping fur or feathers
  - Aseptic scrub of surgical site
  - Providing gentle heat to the animal (circulating water heating pad)
Sterilization of the instruments
  - Begin with a sterile pack of instruments (from an autoclave or gas sterilizer); a bead sterilizer may be used to sterilize instrument tips between surgeries done in a batch on the same day
  - Practicing gentle tissue handling, aseptic handling of instruments, and proper suture/wound closure technique
  - Supportive postoperative care, including providing heat and food/water that is easily accessible
  - Appropriate recordkeeping

For non-survival surgery, aseptic technique does not have to be used. However, instruments and the surgical area should be clean; the incision site should be clipped; and the surgeon should wear gloves.

Checking for Perception of Pain

Before beginning the surgery, you MUST ensure that the animal is adequately anesthetized and cannot feel pain. This can be done by gently but firmly pinching the animal’s toe or footpad. You can pinch with your fingers BEFORE you put on your sterile gloves, or use a sterile hemostat or forceps after you have donned sterile gloves. Take care not to pinch too hard or you could damage the animal’s foot or toe. This should be done periodically to determine that the animal remains anesthetized throughout the procedure.

Note: remember that anything that touches the foot is no longer sterile. You should disinfect your gloves after performing a toe pinch with your fingers. If using forceps, segregate them to avoid contaminating the sterile instruments.

The hand depicted here is ungloved to show the gentle pressure needed for a toe pinch, however, generally gloves will be worn at this point in the procedure.

Supportive Care and Monitoring

Supportive care aims to:
  - Maintain the animal’s physiological status as near normal as possible
Minimize animal pain and distress

Supportive care includes the monitoring of both physiological parameters and analgesia during anesthetic and surgical procedures. Monitoring of vital signs and potential signs of pain should be conducted throughout the procedure and the recovery period.

The photo shows the monitoring of analgesia in a rat (assessing the toe pinch reflex).

Surgical and Anesthesia Problems

Keep in mind that:

- General anesthesia causes disturbances of thermoregulation and other physiological functions. Maintaining body temperature, e.g., via insulating materials and heating pads, is an important objective of supportive care because rats may be unable to properly thermoregulate for some time after some procedures, including anesthesia and surgery.
- During surgery, an animal may experience pain if anesthesia is inadequate at any time during the procedure.
- Postoperatively, the animal may experience pain, discomfort, and distress unless treated with analgesics and appropriate supportive care measures. It is recommended that the first analgesic dose be administered during surgery, or before the animal wakes up from anesthesia. Follow your approved IACUC protocol, and keep documentation on when all analgesic treatments are given.

Due to the interaction of metabolic factors and drug effects that can cause animal mortality, rats should receive good supportive care and frequent monitoring during anesthesia, whether or not the procedure involves surgery.

Supportive Care & Monitoring Procedures

During anesthesia and surgery, the following procedures are strongly recommended. These supportive measures should be included in the animal care and use protocol.

Supportive care:
• Provide a source of warmth to rats from the onset of anesthesia to the end of anesthetic recovery.

• Inject sterile physiological fluid (warmed) to compensate for blood loss during a procedure and depressed fluid intake post-procedure. This should be done in concert with input from the veterinary staff.

Monitoring during anesthesia:

• Analgesia – toe pinch
• Respiration – gross changes in rate, character of breathing
• Color of mucous membrane and skin – blue (poor oxygenation), pale (poor blood perfusion)

The photo shows a rat with the trachea intubated, ready to be connected to a ventilator.
Postsurgical Care

After anesthesia and surgery, the following procedures are recommended:

Monitoring post anesthesia:
  Rats must be monitored until fully recovered from anesthesia as indicated by the ability to ambulate and maintain core body temperature.

Monitoring post procedure:
  - Assess appearance, activity, and behavior as indications of pain and discomfort (see lesson on Detecting Pain and Distress).
  - Assess food and water intake.
  - Provide floor-level access of food and water post procedure if stretching overhead for these items (in the cage wire lid) may be painful.
  - Assess wound healing.

Supportive care and monitoring responsibilities should be detailed in the animal care and use protocol. This should be followed carefully, and all monitoring and treatments documented in a laboratory or animal care record.

Euthanasia

The term euthanasia is derived from Greek and means "good death." Animals should be euthanized when killed for any purpose, including research. To euthanize a rat, you must be trained in the concepts of euthanasia, the method to be used, and the proper handling of rats.

Methods are classified as acceptable or conditionally acceptable, as set by the American Veterinary Medical Association’s document, the AVMA Guidelines on Euthanasia. The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.
The photo shows a CO$_2$ chamber for euthanizing rodents.

Methods
Click the link for each method below for recommendations on its use in euthanizing rats.

Acceptable Methods:
- Barbiturate overdose
- Inhalant anesthetic overdose
- Carbon dioxide (compressed tanks only)
- Carbon monoxide
- Potassium chloride in conjunction with general anesthesia
- Microwave irradiation

Conditionally Acceptable Methods*:
- Nitrogen
- Argon
- Cervical dislocation
- Decapitation

* The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.

Diseases

- Chronic Respiratory Disease
- Coccidiosis
- *Haemobartonella muris* Infection
- Labyrinthitis
- Lice
- Streptococcal Pneumonia
- Mammary Tumors
- Microphthalmia
- Pinworms
- Rat Bite Fever
- Chromodacryorrhea (porphyrin staining - red tears)
- Sialodacryadenits (bulging eyeballs)
- Tapeworms
- Urinary Bladder Worms

References

Federal Laws, Regulations, Policies:


Guidelines:


Texts:

### C. HAMSTER - *Mesocricetus auratus*

<table>
<thead>
<tr>
<th><strong>Life Span</strong></th>
<th>1 year average, 2 years maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight, Adult Male</strong></td>
<td>85-130 grams</td>
</tr>
<tr>
<td><strong>Weight, Adult Female</strong></td>
<td>95-150 grams</td>
</tr>
<tr>
<td><strong>Birth Weight</strong></td>
<td>2 grams</td>
</tr>
<tr>
<td><strong>Breeding Age, Female</strong></td>
<td>2 months, 95-120 grams</td>
</tr>
<tr>
<td><strong>Breeding Age, Male</strong></td>
<td>2 months, 85-110 grams</td>
</tr>
<tr>
<td><strong>Estrus Cycle</strong></td>
<td>3-4 days, polyestrous</td>
</tr>
<tr>
<td><strong>Gestation</strong></td>
<td>15-19 days, 16 average</td>
</tr>
<tr>
<td><strong>Weaning age</strong></td>
<td>21 days, 35 grams</td>
</tr>
<tr>
<td><strong>Begin Dry Food</strong></td>
<td>7-9 days</td>
</tr>
<tr>
<td><strong>Litter Size</strong></td>
<td>4-12, 6-8 average</td>
</tr>
<tr>
<td><strong>Time to Remate</strong></td>
<td>4 days</td>
</tr>
<tr>
<td><strong>Breeding Life, Female</strong></td>
<td>1 year</td>
</tr>
<tr>
<td><strong>Breeding Life, Male</strong></td>
<td>1 year</td>
</tr>
<tr>
<td><strong>Mating</strong></td>
<td>Pairs, 1 M-2 F</td>
</tr>
<tr>
<td><strong>Chromosome Number</strong></td>
<td>44</td>
</tr>
<tr>
<td><strong>Rectal Temperature</strong></td>
<td>37°C - 38°C (98.6°F – 100.4°F)</td>
</tr>
<tr>
<td><strong>Respiration Rate</strong></td>
<td>77 average, 35-135 breaths per minute</td>
</tr>
<tr>
<td><strong>Heart Rate</strong></td>
<td>332 average, 250-500 beats per minute</td>
</tr>
<tr>
<td><strong>Blood Volume % Body Weight</strong></td>
<td>65-80 ml/kg, 6-9%</td>
</tr>
<tr>
<td><strong>Maximum Safe Bleed</strong></td>
<td>5.5 ml/kg</td>
</tr>
<tr>
<td><strong>RBC 1000/CU MM</strong></td>
<td>7.2 average, 4-10</td>
</tr>
<tr>
<td><strong>Hb G/100 ML</strong></td>
<td>16.4 average, 13-19</td>
</tr>
<tr>
<td><strong>PCV ML%</strong></td>
<td>50.8 average, 39-59</td>
</tr>
<tr>
<td><strong>Platelets 1000/CU MM</strong></td>
<td>386 average, 300-570</td>
</tr>
<tr>
<td><strong>WBC 1000/CU MM</strong></td>
<td>8.1 average, 5-11</td>
</tr>
</tbody>
</table>
### Food Consumption

<table>
<thead>
<tr>
<th></th>
<th>8 – 12 g/100g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Consumption</td>
<td>8 – 10 ml/100g/day</td>
</tr>
</tbody>
</table>

### Handling Techniques

**Injuries and Allergies**

Working with hamsters is associated with the following hazards:

**Injuries**

Personnel handling hamsters can be injured by bites from the incisor teeth. Bites often occur because of a lack of knowledge in how to handle, transport, and restrain a hamster. A hamster will bite when startled from sleep, or when frightened, or in pain. Likewise, poor technique in handling, etc., can cause injury to the hamster. Training staff to work effectively and humanely with hamsters is essential to prevent injuries to people and hamsters.

**Allergies**

People can develop an allergy to hamsters after having contact with them for some time. Persons who develop allergic symptoms should seek medical attention and may have to wear specific personal protective equipment (PPE) while working with hamsters. It may be necessary to discontinue working with this species.
Zoonoses

In general, transmission of zoonotic disease from laboratory animals is uncommon because of ongoing efforts by both facility staff and vendors to monitor and improve the health status of animals using reliable health surveillance programs. Experimentally infected animals are a source of zoonotic transmission to humans. Infected cell lines, animal tissues, and animal tissue products can also be sources. Health surveillance programs, routine sanitation, and personal protective equipment have important roles in preventing zoonoses.

Hamsters can be a reservoir of the following infectious agents which are transmissible to people:

Viruses:

- Lymphocytic choriomeningitis virus (LCM)

Bacteria

- *Leptospira* spp.

Protozoa

- *Giardia* spp.

Helminths

- *Rodentolepis nana*
Housing

Your protocol application may ask you which type of housing you may need for your hamsters. There are important considerations in the selection of animal housing that affect the welfare of your animals. For the appropriate cage space per animal, refer to the minimum requirements specified by the Animal Welfare Regulations.

Flooring

Hamsters are generally housed in shoebox cages with a solid floor covered with bedding material that absorbs liquid wastes. Bedding has been shown to be preferred by rodents for resting, and it is considered to provide them with comfort, warmth, and the opportunity to burrow. This type of flooring is well suited for breeding because pups are better protected from chilling.

Most institutions use microisolator cages (filter cage top and solid bottoms) under barrier conditions or in open-topped cages (no filter top) under conventional conditions. Hamster caging must be secure, e.g., with tightly fitting wire lids, because hamsters are prone to dislodge cage tops and escape through narrow spaces. Hamsters have a solitary nature and so may prefer to be housed individually.

Acclimation

Upon arrival to your facility, your hamsters should have an acclimation period before they are used in research studies. This period of time allows animals to adapt to a new environment. Effects of transportation stress may include alterations in various blood parameters, immune cell function, food intake, and animal behavior. The period of time necessary for biological stabilization will depend on the parameters to be studied. The typical acclimation period for hamsters would be 3 to 7 days. Refer to your institution’s attending veterinarian for recommendations that are appropriate for your project.
Example Alternatives Search:

For citations on acclimatization (alternate term for acclimation) on hamsters, please click on the following search template to execute your search online:

PubMed

- **hamsters AND acclimatization**

**Quarantine**

Routine quarantine, if required, may prolong the holding of your animals in special facilities. Quarantine aims to prevent transmission of diseases between new animals and established colonies, so diagnostic tests may be performed during this period.

Acclimation and quarantine periods run concurrently, although they serve different purposes. Many institutions do not allow experiments on animals while quarantined.

Quarantine procedures are specific to the institution receiving the animals based on the origin of the animals, institutional policy, and health status of the animal colonies at the institution.

**Detecting Pain and Distress**

If your proposed study involves a painful procedure, the protocol form may ask for a method of assessing if the hamsters are experiencing pain or distress.

Assessing pain and distress in hamsters is difficult at times because hamsters, like many other species, commonly conceal outward signs of moderate pain and distress. In this case, the behavioral changes that reveal a hamster's pain and distress may be subtle and detectable only with specialized behavioral methods.
Clinical Signs

Severe pain and distress causes overt clinical signs in hamsters. Laboratory staff working with hamsters should be trained to recognize these abnormalities in:

- Activity level
  - Depression
  - Lethargy
  - Extended sleep
- Behavior
  - Aggressiveness
  - Self-trauma
  - Uncoordinated movements/ataxia
  - Vocalization (chatter or screech)
- Appearance
  - Ruffled fur
  - Ocular discharge
  - Hunched posture
  - Pale mucous membranes
  - Soiled anogenital area
- Vital signs
  - Respiratory distress
  - Hyperthermia
  - Hypothermia
- Body Condition
  - Weight loss
  - Emaciation
  - Dehydration
- Intake
  - Reduced intake of food and water
Chronic Pain & Distress

A chronic state of pain or distress may be more subtle and difficult to detect. A good knowledge of the animal’s normal appearance and behavior is especially important to recognize chronic pain or distress.

For methods on assessing and alleviating pain and distress in rodents, please refer to the course, "Post Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress." The methods described are pertinent to all laboratory rodents including hamsters.

Species/Strains

The following is a list of hamster species used in biomedical research. The Syrian and Chinese hamster species are the most commonly used.

- Golden hamster or Syrian hamster, Mesocricetus auratus. (adults 120 g)
- Chinese or Striped hamster, Cricetulus griseus (adults 30-35 g)
- Armenian hamster, Cricetulus migratorius (approx. 45 g)
- European hamster, Cricetus cricetus (adults approx. 350 g)
- Siberian or Djungarian hamster, Phodopus sungorus (adults 30-50 g)

The image below shows a Chinese hamster (left), an Armenian hamster (middle), and a Syrian hamster (right).

Outbred Stocks and Inbred Strains

Most hamsters are outbred stocks, meaning that individuals have a high degree of heterozygosity and phenotypic variability.

Inbred (isogenic) strains of the Syrian hamster (Mesocricetus auratus) are used for cardiomyopathy studies. These strains are designated:

- CHF148 (spontaneous disease model)
- CHF146 (control)
Inbred strains are typically used for finely controlled studies that capitalize on genetic isogenicity. Inbred strains with characteristics of human diseases or physiological conditions are generally preferred models for biomedical research.

Please check with your animal resource department for information on vendor choices as animal source affects animal health status.

**Biological Features**

Though hamsters share many anatomical and physiological features with humans, hamsters have many unique biological characteristics.

In the image at right, a hamster's cheek pouch is everted to show the mucosal surface.

Knowledge of species-specific characteristics is helpful to effectively manage these animals and to plan experimental procedures for their use. Researchers should be aware of the following practical features of hamster anatomy and biology.

**Biting**

Hamsters are inclined to bite when startled, frightened, or in pain. A common cause for biting is handling the animal while it is asleep in its cage. Hamsters may sleep very deeply, and a sudden awakening may elicit an aggressive response. It is important to carefully wake the animal and make it aware of your intention to handle it. If a hamster is prone to bite an approaching hand, a small container like a can may be used to scoop up the hamster and remove it from the cage.
Escapism

During their active period (at night), hamsters may escape if they determine that their cage is not secure, e.g., via a loosely fitting wire lid. They are adept at chewing through cage materials, including plastic. Therefore, cages must be made secure with the use of strong materials and tightly fitting cage components.

Feeding behavior

Hamsters prefer to be fed on the cage floor. With their blunt noses, they are less able to grasp rodent food blocks overhead in the feed hopper of the cage wire lid. However, with wire mesh that is sufficiently spaced for access to the food blocks, hamsters can pull the food into their cage. Contamination of food on the floor with wastes is less of a concern in hamsters because they expel their wastes and store their food in separate sites.

Hamsters tend to hoard food in their cage and also in their cheek pouches. Refer to the section on cheek pouches for biological features of these structures.

Nocturnalism

Hamsters are very active at night and spend most of their day asleep. During the night, they will explore their cage and make use of enrichment devices, if available. For example, hamsters will run for long periods on exercise wheels.

Social characteristics

Hamsters are solitary animals by nature and may prefer to be housed separately. If grouped since weaning, cage mates may live peacefully together, although stress has been reported in group-housed hamsters. Fighting may result if unfamiliar adults are mixed together. Females, especially Chinese hamsters (*Cricetulus griseus*), are more prone to be aggressive toward strangers, except when presented with a male during estrus.

Cheek pouches

Hamsters have cheek pouches that extend along the head to the neck area. Cheek pouch dimensions have been reported to be 35-40 mm by 4-8 mm by 20 mm long. The hamster uses these pouches to carry and store food. A female also uses her cheek pouches to carry or conceal her young. Often, persons unfamiliar with this feature will report that the hamster has swellings or lesions in the throat area when they have observed a hamster with full pouches.

A cheek pouch is a muscular sac lined with stratified squamous epithelium. The lining of the cheek pouch may be everted and examined in an anesthetized hamster. This epithelium is thin-walled, highly distensible, highly vascular, and largely devoid of lymphatic tissue except for some lymph vessels. Due to the lack of lymphatic tissue, the cheek pouches are
immunologically privileged and have been used as sites for tumor induction and implantation. The hamster cheek pouch model is also widely used in drug safety and toxicology testing.

Teeth and caries

Hamsters have incisors that are open-rooted, meaning that these teeth grow continuously throughout adult life. A diet of soft foods, i.e., in liquid or powder form, or a developmental jaw malformation will cause tooth overgrowth. Staff must be alert to detect any signs of this condition and to provide appropriate treatment.

The molars retain fine particles of food and are prone to dental caries (cavities), making the hamster a model for dental research.

The dental formula is 1/1 incisors and 3/3 molars. Like other rodents, hamsters lack canines and premolars.

Scent glands

Sebaceous scent glands are located on the dorsal flank (hip region) along the spine. These are present in both sexes, although they are more prominent in males. When the hair is removed, these glands are visible as dark areas of the skin. These glands are stimulated by androgens. The secretions are used to mark territory and are involved in sexual behavior.

High rate of metabolism – impact on drug clearance

The hamster’s high rate of metabolism produces a rapid clearance of drugs from the body. Drugs administered at dose rates used in larger species (with lower metabolic rates) would reach lower blood concentrations and exert less pharmacological effect in the rat. As a result, hamsters should receive drug doses that have been scaled to the hamster’s metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

In general, hamster-specific dose rates have been determined for drugs that are commonly used in animal research, such as anesthetics, analgesics, sedatives, and antibiotics. Investigators are advised to obtain hamster dose rates from laboratory animal references or from their institution’s veterinary staff.

High surface area – impact on hypothermia

Hamsters have a large body surface area (relative to body volume). As a result, hamsters are vulnerable to profound hypothermia under conditions of sedation and anesthesia. Sedation and anesthesia induce hypothermia due to drug effects on the hypothalamus and a rapid drop in core body temperature. If surgery is being performed, additional heat is lost by convection from an open incision during surgery.
Hamsters should have a source of warmth throughout a procedure that lowers their body temperature (e.g., anesthesia, surgery) and afterwards until they recover the ability to thermoregulate themselves.

**Pseudohibernation**

Hamsters do not undergo a true hibernation but a pseudohibernation instead. The distinction is that they remain sensitive to touch and so are readily aroused from their hibernatory state. Under conditions of shortened day length, cool temperatures (8 °C, 48 °F), less light, and social isolation, hamsters hoard food and enter into pseudohibernation. During this state, the hamster has a decreased body temperature, low respiration rate, and a low heart rate. The animal will arouse every two to three days with short periods of normal alertness and activity.

**Inability to vomit**

Hamsters do not vomit because they lack the neurophysiological mechanisms for emesis. Therefore, presurgical fasting is not necessary in hamsters, as it is for nonrodent species.

**Coprophagy**

In hamsters, herbaceous foodstuffs are broken down by microbial action in the cecum, which is a large organ in the hamster. To assimilate the microbial byproducts of digestion, the hamster regularly eats its own feces, a habit known as coprophagy. Hamsters will consume feces directly from the anus. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the animal.

**T-cells**

Hamsters lack suppressor T cells and have atypical cytotoxic T cells.

**Procedures for Injections and Blood Collection**

**Volume Recommendations**

Volume recommendations for intravenous fluid administration and blood collection in adult Syrian hamsters\(^a\) are given below. These recommendations are currently under review. You should consult your facility veterinarian if there is any question about the appropriate volume for a particular procedure.

<table>
<thead>
<tr>
<th>IV fluid volume (ml)</th>
<th>Total Blood Volume (ml)</th>
<th>Safe Bleed Volume (ml)(^a)</th>
<th>Bleed-out Volume (ml)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>max. acute admin.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
a Removing greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids to replace the volume of blood collected. In addition, monitor the animal’s hematocrit for anemia.

b Animals should be exsanguinated only under anesthesia.

From:


**Peripheral Blood Vessels**

Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 23 to 27 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Vessels</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Lateral saphenous vein (lateral tarsal vein) | 1. Accessing the lateral saphenous vein:  
   o Does not require anesthesia.  
   o May be aided by sedation because vein visibility is enhanced by peripheral vasodilation (drug effect).  
   o May be aided by sedation to reduce animal struggling due to distress.  
   2. Blood collection from the lateral saphenous vein does not involve cannulation of the vein lumen. Instead, the vein is punctured percutaneously and blood is passively collected as it pools on the skin. |
| Jugular vein                        | Performed under anesthesia because of restraint method and the need for animal immobilization. |
| Cardiac puncture  
  Carotid artery                    | 1. These two methods require anesthesia. |
### Abdominal vena cava

- Performed as a terminal procedure under anesthesia.
- Vessel access involves a ventral midline incision and reflection of intestines.

### Abdominal aorta

- Most often allowed only as a terminal procedure.
- Check with your institution for guidelines on these routes of blood collection.

### Retroorbital sinus

- Retroorbital puncture is controversial because of the risk of injury to the optic nerve and other nearby structures.
- This method is considered to be painful and may cause blindness.
- Generally requires anesthesia.
- Topical ophthalmic anesthetic is recommended post-procedure.

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**Nonvascular Injections**

Below are the nonvascular routes of injection that are commonly used in hamsters. Included are volume recommendations for the safe administration of fluids acutely in adults (Syrian hamster, average 120 g). Recommended needle sizes are 23 to 27 gauge; larger needles may be necessary for injecting viscous materials.

- **Subcutaneous (SQ or SC)** - 3-4 ml in scruff
- **Intraperitoneal (IP)** - 3-4 ml
- **Oral (PO)** - 20 ml/kg
- **Intradermal (ID)** - 0.05 ml/site
- **Intramuscular (IM)** - 0.1 ml per site
- **Cheek pouch** - 0.1 ml instilled into wall of everted pouch.

From:


Example Alternatives Search

For alternatives of injection and blood collection methods in hamsters, please refer to the following search templates:

PUBMED:

- hamsters AND injections
- hamsters AND (blood AND specimen AND collection/methods)

Website:

- Browse research guidelines of institutional IACUCs, associations, and councils on www.IACUC.org

Analgesics, Sedatives, and Anesthetics

Diseases

Antibiotic Toxicity  Pinworms
Demodectic Mange  Protozoa
Hibernation  Tapeworms
Impacted Cheek Pouch  Vaginitis
Lymphocytic Choriomeningitis  Wet-tail

References

Federal Laws, Regulations, Policies:

5. USDA Animal and Plant Health Inspection Animal Care Policy Manual Policy #11 - Painful/Distressful Procedures
6. U.S. Government Principles For The Utilization And Care Of Vertebrate Animals Used In Testing, Research, And Training, Interagency Research Animal Committee
Guidelines:


Texts:

D. **RABBIT - *Oryctolagus cuniculus***

<table>
<thead>
<tr>
<th>Trait</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>6 yrs avg, 15 yrs max</td>
</tr>
<tr>
<td>Weight, Adult Male</td>
<td>4-5.5 kg</td>
</tr>
<tr>
<td>Weight, Adult Female</td>
<td>4.5-6.5 kg</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>100 grams</td>
</tr>
<tr>
<td>Breeding Age, Female</td>
<td>5-6 months, 4.5 kg</td>
</tr>
<tr>
<td>Breeding Age, Male</td>
<td>6-7 months, 4 kg</td>
</tr>
<tr>
<td>Estrus Cycle</td>
<td>Polyestrous, Induced</td>
</tr>
<tr>
<td>Gestation</td>
<td>30-32 days, 31 avg</td>
</tr>
<tr>
<td>Weaning age</td>
<td>8 weeks, 1.8 kg</td>
</tr>
<tr>
<td>Begin Dry Food</td>
<td>16-18 days</td>
</tr>
<tr>
<td>Litter Size</td>
<td>1-18, 8 avg</td>
</tr>
<tr>
<td>Time to Remate</td>
<td>35-42 days</td>
</tr>
<tr>
<td>Breeding Life, Female</td>
<td>1-3 year</td>
</tr>
<tr>
<td>Breeding Life, Male</td>
<td>1-3 year</td>
</tr>
<tr>
<td>Mating</td>
<td>Pairs, 1 M, 6-10F</td>
</tr>
<tr>
<td>Chromosome Number</td>
<td>44</td>
</tr>
<tr>
<td>Rectal Temperature °C</td>
<td>39.5</td>
</tr>
<tr>
<td>Respiration Rate</td>
<td>40 avg, 35-56</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>260 avg, 205-308</td>
</tr>
<tr>
<td>Blood Volume % Body Weight</td>
<td>45-70 ml/kg, 6-7%</td>
</tr>
<tr>
<td>Maximum Safe Bleed</td>
<td>7.7 ml/kg</td>
</tr>
<tr>
<td>RBC 1000/CU MM</td>
<td>6.5 avg, 5-8</td>
</tr>
<tr>
<td>Hb G/100 ML</td>
<td>13.5 avg, 8-17</td>
</tr>
<tr>
<td>PCV ML%</td>
<td>40.8 avg, 31-50</td>
</tr>
<tr>
<td>Platelets 1000/CU MM</td>
<td>468 avg, 250-750</td>
</tr>
<tr>
<td>WBC 1000/CU MM</td>
<td>8.6 avg, 3-12.5</td>
</tr>
</tbody>
</table>
Injuries and Allergies

Working with rabbits is associated with the following hazards:

Injuries

Personnel handling rabbits can be injured by bites (incisors) or scratches (especially by the hind feet). Generally, these are caused by a lack of knowledge in how to handle, transport, and restrain a rabbit. Likewise, poor technique in handling, etc., can cause injury to the rabbit. Training staff to work effectively and humanely with rabbits is essential to prevent injuries to people and rabbits.

Allergies

Everyone working with laboratory animals should be enrolled in their institution’s occupational health and safety program. This program will help you monitor any development of allergies, since it is possible for people working with rabbits to develop allergies after some time.

It is essential that workers exposed to rabbits wear proper personal protective equipment (PPE), including scrubs or disposable gowns, gloves, and shoe covers. If you are already allergic to rabbits a respirator (such as an N95 type) can be worn to protect from airborne allergens. Persons who develop allergy symptoms should seek medical counseling from the occupational health and safety program. In severe cases, personnel may have to discontinue working with this species.

Zoonoses

Zoonoses are diseases transmitted by animals to humans.

In general, transmission of zoonotic disease from laboratory animals is uncommon because of ongoing efforts by both facility staff and vendors to monitor and improve the health status of animals using reliable health surveillance programs. Experimentally infected animals are a source of zoonotic transmission to humans. Infected cell lines, animal tissues,
and animal tissue products can also be sources. Also, contact with wild rabbits in field research may expose humans to zoonotic agents carried by this species. Health surveillance programs, routine sanitation, and personal protective equipment have important roles in preventing zoonoses.

Rabbits can be a reservoir of the following infectious agents which are transmissible to people.

**Bacteria:**

- *Francisella tularemia*
- *Leptospira* spp.
- *Yersinia pseudotuberculosis*

**Fungi:**


**Housing**

Rabbits should be housed in pairs or groups in cages or pens. Adult animals may be difficult to introduce for pair housing. Follow your institutional social housing SOP.

Minimum cage size requirements are specified by the Animal Welfare Regulations. Although metal cages can be used for rabbits, plastic cages may be preferred because they provide the animals a quieter, warmer environment. Rabbits are intelligent animals that easily become bored when caged. Therefore they should be offered enrichment when the study allows.
Enrichment

Rabbits can be playful and will use a variety of environmental enrichment items. They seem to enjoy objects that they can nudge and toss around their cage, as well as hanging chains, triangles, or rattles. Nylon chew bones and rubber balls with bells are also an option.

Timothy hay cubes or autoclaved hay can be provided as a food supplement. Rabbits will also eat a variety of fresh fruit, vegetables, and cereal.

Housing and Disease Transmission

For reasons of disease transmission, rabbits should be separated from contact with:

- Other species, including guinea pigs, because of the potential for contagion across species. Example: *Bordetella bronchiseptica* is transmissible from rabbits to guinea pigs.
- Rabbits of different pathogen status. Specific pathogen free rabbits are at risk for infections and parasitic infestations transmitted from rabbits obtained from conventional sources.

Please refer to your animal facility staff for procedures to protect the health status of your rabbits.
Acclimation

Upon arrival to the facility, rabbits should have an acclimation period before they are used in research studies. This period of time allows animals to adapt to a new environment. Effects of transportation stress include alterations in various blood parameters, immune cell function, food intake, and behavior. The period of time necessary for biological stabilization will depend on the parameters to be studied. Refer to your institution’s attending veterinarian for recommendations that are appropriate for your project. Typically, acclimation periods range from 4 days to 1 week.

Example Alternatives Search:

For citations on acclimation for rabbits, please click on the following search template to execute your search online:

NAL Catalog (Agricola)

- rabbits AND acclimation

Quarantine

Routine quarantine procedures, if required, may prolong the holding of your animals in special facilities. Quarantine procedures are specific to the institution receiving the animals based on the origin of the animals, institutional policy, and health status of the animal colonies at the institution. Quarantine aims to prevent transmission of diseases between new animals and established colonies.

Acclimation and quarantine periods run concurrently, although they serve different purposes. Many institutions do not allow experiments on animals while quarantined.
Biological Features

Though rabbits share many anatomical and physiological features with humans, rabbits have many unique biological characteristics. Knowledge of species-specific characteristics is helpful to effectively manage these animals and to plan experimental procedures for their use.

The photo shows a rabbit with jaw malalignment and incisor overgrowth.

Researchers should be aware of the following practical features of rabbit anatomy and biology. Click on the following items for a brief description and some practical tips.

Behavior

Normal Behavior

Rabbits are very intelligent animals, a fact that is often overlooked when they are housed alone in cages. Rabbits often have a shy temperament and can be easily intimidated. When frightened, rabbits may freeze or hunch down, stomp their rear feet, or they may panic and bolt. However, if feeling defensive when approached in the cage, a rabbit may attack and bite. Though often shy, rabbits can be forceful and will make noise by throwing toys around. Therefore, rabbits can benefit from being housed in pairs and being provided with toys and objects to manipulate.

Aggression

When you work with rabbits, be gentle and quiet; don’t make sudden movements or speak loudly. If confronted with an aggressive rabbit in a cage, place a towel over the animal to cover it completely. This allows you to safely scoop the covered rabbit in your arms and remove it from the cage while supporting the hind end. Once out of the cage, most aggressive rabbits are easy to handle and should be treated gently like any other rabbit.
Gastrointestinal

Teeth

Rabbits have teeth that are open rooted, meaning that these teeth grow continuously throughout adult life. Particularly if the jaws are anatomically malaligned, a rabbit’s teeth have the potential to overgrow; although the first incisors are most likely to overgrow, so may the second incisors and the cheek teeth. Staff must be alert to detect any signs of this condition and to provide appropriate treatment.

Inability to vomit

Rabbits do not vomit because they lack the neurophysiological mechanisms for emesis. Presurgical fasting is not necessary in rabbits, as it is for larger laboratory species (carnivores and omnivores).

Dietary Fiber

Rabbits are herbivores and hind gut fermenters, i.e., they digest plant materials in the cecum. Rabbits need higher dietary fiber than do rodents, especially for general maintenance (~22.5%). This is helpful also to reduce obesity in caged rabbits, to promote better digestion, to reduce the incidence of gastrointestinal disorders and inappetance. Supplementing with alfalfa or other hays is helpful to promote normal gut motility.

Low fiber diets will alter the production of cecotropes (a normal type of feces in rabbits) because of hypomotility of the hindgut and a prolonged retention time of material in the cecum. Hypomotility may lead to diarrhea or cecal impaction.

Obesity

Caged rabbits fed ad libitum commonly become overweight. To prevent obesity, rabbits are generally fed a high fiber diet in restricted amounts.

Coprophagy and digestive strategy

Rabbits are herbivores that have a unique strategy for digesting plant materials in the cecum. The rabbit excretes dietary fiber without expending energy to digest it. In the large intestine, dietary fiber is separated from the digesta and excreted as pelleted feces, which accumulate on the cage pan. The nonfiber material (fluids and small particles) are returned to the cecum for further digestion and fermentation. After a period of hours (and generally at night), the cecum expels some of its contents, which are passed as a type of feces known as cecotropes. The rabbit consumes cecotropes directly from the anus. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the rabbit.

Elizabethan or cervical collars are sometimes used on rabbits to prevent them from reaching and disturbing/dehiscing a surgical incision. Since a collar prevents the rabbit from bending over to reach the posterior area of the body, it also prevents the animal from consuming the expelled
cecotropes. Therefore, the use of a collar should not be an automatic decision, and if used, a collar should be removed at the earliest opportunity.

Enteropathy

Rabbits are extremely vulnerable to microbial imbalances in the cecum and colon. Factors that can contribute toward developing an enteropathy are:

- **Infectious agents** - *Clostridium spiroforme* and *E. coli*. Destabilization of gut microbial populations (e.g., due to pH changes or antibiotic treatment) leads to a proliferation of *E. coli* and *Clostridium*.
- **Dietary factors** - Low fiber diets cause cecocolonic hypomotility. When fiber content is low, digestible carbohydrate overload causes an enterotoxemia. Associated factors are acidification of the cecum, overgrowth of *E. coli* and *Clostridia*, and reduced volatile fatty acid production.
- **Stress** – due to inhibition of intestinal motility. Common stress factors are environmental temperature change, dietary change, and transportation.

Infectious

SPF vs non-SPF

Specific pathogen free (SPF) rabbits are produced in isolation from specific rabbit pathogens and parasites. Rabbit sources vary in the pathogens that are absent from their animal stock, but SPF rabbits are commonly free of:

**Bacteria** -


**Viruses** -

Rabbit hemorrhagic disease virus, rabbit pox virus, rabbit rotavirus.

Because many facilities may have both SPF and non-SPF rabbit colonies, it is important that all staff comply with facility procedures aimed at preventing contamination of the SPF rabbits.
Sensitivity to antibiotics

Antibiotics, especially those with a Gram-positive spectrum of activity like the penicillin family, may cause a toxic shock syndrome in rabbits. Antibiotic that reaches the intestine, e.g., by biliary excretion, kills some of the intestinal flora and allows an overgrowth of resistant *Clostridium* species. With proliferation of these species, clostridial toxin is produced and released. The animal develops a hemorrhagic cecitis and colitis. The rabbit may present with diarrhea initially and die very quickly.

Metabolism

Albinism

The most common research rabbit is the New Zealand White, which is an albino breed. Albinism in rabbits is an inherited disorder of melanin metabolism caused by the lack of the enzyme tyrosinase, which has an impact both on melanocytes and neurons. Neuronal morphological abnormalities and functional impairments involve the following sites: medial vestibular nucleus, cochlear nuclei, and retina. Studies comparing albino and pigmented animals have shown differences even in pharmacotoxic kinetics in these tissue areas.

In the rabbit, there is a graded series of alleles at the C locus providing a step-wise reduction in color. The genetics of albinism in rabbits has been used to study the physiological effects of albinism.

Drug clearances

The rabbit has a higher rate of metabolism than larger species, such as dogs, but less than the laboratory rodents, such as mice and rats. Compared to these species, the rabbit has an intermediate rate for drug clearance from the body. Rabbits should receive drug doses that have been scaled to the rabbit's metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

In general, rabbit-specific dose rates have been determined and are widely published for drugs that are commonly used in animal research, such as anesthetics, analgesics, sedatives, and antibiotics. Investigators are advised to obtain rabbit dose rates from laboratory animal references or from their institution's veterinary staff.

Respiratory System

- Endotracheal Intubation

Rabbits have a narrow oral cavity (glottis) that restricts visibility of the larynx for endotracheal intubation. A visual method of intubation is difficult because of the limited space for insertion of a laryngoscope. However, the blind method of intubation is easy due
to the simple anatomy of the rabbit’s oropharynx and laryngopharynx. Rabbits should be routinely intubated in the trachea when consistent with the anesthetic regimen.

Skeletal System

- Spinal fracture

Rabbits housed in cages have bony hypoplasia and may have osteoporosis due to inactivity imposed by living in the restricted space of a cage. As a result, laboratory rabbits are known for the fragility of their bones. The lumbar spine is most prone to injury and fracture. A forceful kick by the strong, muscular hind limbs is sufficient to cause a lumbar fracture (most commonly at the L7-S1 junction). Rabbits must be carefully handled when transported or restrained to prevent them from panicking and kicking out with their hind limbs. Rabbits with a spinal fracture will present as splayed and paralysed in rear limbs.

Note: This may be masked by the rabbit’s habit of sitting motionless.

Detecting Pain and Distress

Assessing Pain and Distress

If your proposed study involves a painful procedure, the protocol form may ask for a method of assessing whether the rabbits are experiencing pain or distress.

Assessing pain and distress in rabbits is difficult at times because rabbits, like many other species, commonly conceal outward signs of even moderate pain and distress. In this
case, the behavioral changes that reveal a rabbit’s pain and distress may be subtle and detectable only with specialized behavioral methods.

Clinical Signs

Rabbits may also exhibit overt clinical signs of pain and distress, the more so when pain is more intense. Laboratory staff working with rabbits should be trained to recognize these abnormalities in:

- Activity level
  - Hypoactivity
  - Lethargy
  - Restlessness
- Behavior
  - Vocalization
  - Self-trauma
  - Aggressiveness
  - Ataxia
- Appearance
  - Protruded third eyelid (nictitating membrane)
  - Hunched posture
  - Cyanosis
  - Pale mucous membranes
  - Fecal-stained fur
- Vital signs
  - Respiratory distress
- Body condition
  - Weight loss
  - Emaciation
  - Dehydration
- Intake:
  - Reduced intake of food and water

Chronic Pain and Distress

A chronic state of pain or distress may be more subtle and difficult to detect. A good knowledge of the animal’s normal appearance and behavior is especially important to recognize chronic pain or distress.
Procedures for Injections and Blood Collection

Volume Recommendations

Below are volume recommendations for acute intravenous fluid administration and blood collection in rabbits.

<table>
<thead>
<tr>
<th>IV fluid volume (mL)</th>
<th>Tot. blood volume (mL)</th>
<th>Safe bleed volume (mL)^a</th>
<th>Bleed-out volume (mL)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL/kg</td>
<td>57-65 mL/kg</td>
<td>7.7 mL/kg</td>
<td>31-310 mL</td>
</tr>
</tbody>
</table>

^a Removing greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids to replace the volume of blood collected. In addition, monitor the animal’s hematocrit for anemia.

^b Animals should be exsanguinated only under anesthesia.

From:


Peripheral Blood Vessels

Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 23 to 25 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Route of Vascular Access</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear vein</td>
<td>1. These methods may be performed without sedation, although sedation is helpful for vasodilation and chemical restraint.</td>
</tr>
<tr>
<td>Ear artery</td>
<td>2. Topical anesthetic formulations may be applied to produce a local anesthesia.</td>
</tr>
</tbody>
</table>
1. These methods are typically used with sedation or anesthesia for chemical restraint.
2. If these veins must be accessed via a surgical incision, the following methods will be required: general anesthesia, aseptic technique, and peri-operative monitoring.

1. Cardiac puncture requires anesthesia.
2. Cardiac puncture is most often allowed only as a terminal procedure. Check with your institution for guidelines on this route of blood collection.

**Nonvascular Injections**

Below are the nonvascular routes of injection that are commonly used in rabbits. Included are volume recommendations for the acute administration of fluids. Recommended needle sizes are 23 to 25 gauge; larger needles may be necessary for injecting viscous materials.

**Subcutaneous (SQ or SC)**
30 - 50 mL (scruff, flank) - limit 20 mL per injection site

**Intraperitoneal (IP)**
50 - 100 mL

**Oral (PO)**
5 mL

**Intradermal (ID)**
0.1 mL/site

**Intramuscular (IM)**
0.5 - 1.0 mL per site

From:

Polyclonal Antibody Production: Antigens & Adjuvants

When using any animal species for polyclonal antibody production, the issues below should be addressed within the animal protocol. For more detail, refer to the Institute for Laboratory Animal Research publication, ILAR Journal Volume 37(3) Adjuvants and Antibody Production, 1995.

Antigen Preparation

The antigen preparation should be free of extraneous microbial contamination and byproducts such as polyacrylamide gel. The protocol should describe how the antigen-adjuvant emulsion will be prepared.

Adjuvant Used

If the use of Freund’s complete adjuvant (FCA, also called CFA) is proposed, your IACUC may require justification of this choice of adjuvant. FCA has been associated with granulomatous inflammation, focal necrosis, ulceration of skin, fistulous tracts, muscle atrophy, self-induced trauma, hypersensitivity reactions, and weight loss. The USDA states that the injection of FCA may cause more than momentary or slight pain. This means that FCA injections might place an animal into USDA pain category D (painful/stressful but relieved), requiring the use of post-injection analgesics. Check with your IACUC to determine your institution’s policy.

Recommendations for FCA from the Institute of Laboratory Research are as follows:

1. FCA should be used only once, usually for the initial immunization.
2. Formulations of FCA should not exceed 0.1 mg dry mycobacterial cell mass/mL.
3. Less inflammatory alternatives to Freund’s adjuvant are available and should be considered.
Polyclonal Antibody Production: Immunizations

Booster Frequency

In common booster schedules, the initial and subsequent immunizations are spaced at intervals of two to three weeks (minimum). Booster immunizations may be delayed if significant inflammatory reactions are still present from the initial immunization.

Injection Site Selection and Preparation

Excerpted from the ILAR Journal, vol 37, issue 3 (Institutional Policies and Guidelines on Adjuvants and Antibody Production): "Anatomic sites used for grasping, handling, or restraint...should be avoided when possible. Extension of granulomatous inflammation into the spinal cord following inadvertent injection of a FCA-antigen mixture into the paraspinal musculature has been associated with posterior paresis in guinea pigs (Kleinman et al., 1993). Care therefore should be taken when making injections near the dorsal spinal column. Granulomas can also be noted in other organs after injections with FCA (Schiefer and Stunzi, 1979)."

Post-injection Observations

Your animal protocol should describe how animals will be monitored for post-injection lesions and how affected animals will be treated.

Analgesics, Sedatives, and Anesthetics

Because rabbits have a high rate of metabolism, drugs are rapidly eliminated from their bodies. Dose rates appropriate for larger species produce ineffective drug concentrations in the bloodstream when used in rabbits.

These agents are described below and rabbit dose rates for common drugs and drug regimens are provided. If you need to use other drug agents, check with your institution's veterinary staff for assistance in determining a dose rate appropriate for use in rabbits.
• Analgesics:

Available in two drug types – the opioids and the nonsteroidal anti-inflammatory drugs (NSAIDs). The rapid clearance of many of these drugs in rabbits results in the need for an increased frequency of administration.

• Sedatives:

Sedatives have no effect on consciousness, the perception of pain or other sensations. When combined with general anesthetics, the result is a balanced anesthesia where muscle relaxation, unconsciousness, and analgesia are enhanced.

• Sedatives + Analgesia:

Some sedatives also have analgesic effects. When combined with general anesthetics, a balanced anesthesia is attained, and these sedatives enhance analgesia through specific effects.

• Anesthetics:

Because rabbits metabolize drugs so rapidly, many anesthetic agents have brief durations of effect. An anesthetic regimen should be chosen to match the duration of drug effects with the length of the procedure. In particular, short acting agents (and regimens) should be not be used for long procedures because repeat drug administrations, necessary to prolong anesthesia, will produce uneven blood concentrations and therefore periodically inadequate anesthesia. For long procedures, gaseous anesthesia is the often the most practical method to sustain uniformly adequate levels of anesthesia.

Surgery

Definitions

Aseptic technique should be used when performing major survival surgery on rabbits. This is required by the Animal Welfare Act (federal law). The standards described here are consistent with the Guide for the Care and Use of Laboratory Animals.

• Survival surgery means that the animal regains consciousness after anesthesia. In nonsurvival surgery, the animal is euthanized before recovery from anesthesia.

• Major surgery means penetrating and exposing a body cavity such as the chest or abdomen; or producing substantial physical or physiological impairment.
**Major Survival Surgery Requirements**

If you will be performing major survival surgery on rabbits, federal requirements are that:

- The surgery must be carried out in a dedicated surgical facility.
- The surgeon must wear a cap, mask, sterile gown, and gloves.
- The incision site is appropriately clipped, scrubbed, disinfected, and draped.
- Instruments and surgical materials are sterile.
- The animals receive proper supportive care and monitoring for anesthesia and vital signs through the procedure and afterward.

Note: Because rabbits cannot vomit, it is unnecessary (and usually not advised) to fast them prior to surgery.
**Minor Procedures**

Minor survival surgery also requires the use of sterile instruments and aseptic technique. Minor surgery does not expose a body cavity and causes little or no physical impairment. Minor surgery on rabbits consists of procedures such as peripheral vessel cannulation, wound suturing, and percutaneous biopsy.

If you will be performing nonsurvival surgery, it may not be necessary to follow all the techniques outlined above for major survival surgery. According to the *Guide* "at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean" (p 118).

**Supportive Care and Monitoring**

**Overview**

Supportive care aims to:

- Maintain the animal's physiological status as near normal as possible.
- Minimize animal pain and distress.

Supportive care includes the monitoring of both physiological parameters and analgesia during anesthetic and surgical procedures. Monitoring of vital signs and potential signs of pain should be conducted throughout the procedure and the recovery period.
**Key Concerns**

Keep in mind that:

- General anesthesia causes disturbances of thermoregulation and other physiological functions. Some animals are unable to properly thermoregulate for a while after some procedures, including anesthesia and surgery.
- Warming devices (e.g., heated tables and pads) are recommended for routine use to maintain the animal's body temperature.
- During surgery, the animal may experience pain if anesthesia is inadequate at any time during the procedure.
- Postoperatively, the animal may experience pain, discomfort, and distress unless treated with analgesics and appropriate supportive care measures.

Due to the interaction of metabolic factors and drug effects that can cause animal mortality, rabbits should receive good supportive care and monitoring during anesthesia, whether or not the procedure involves surgery.

**Procedures During Anesthesia and Surgery**

During anesthesia and surgery, the following procedures are strongly recommended.

**Supportive care:**

- Provide a source of warmth to a rabbit from the onset of anesthesia to the end of anesthetic recovery.
- Infuse sterile physiological fluid (warmed) to compensate for blood loss during a procedure and depressed fluid intake post-procedure. This should be performed in concert with input from the veterinary staff.

These supportive measures should be included in the animal care and use protocol.

**Monitoring during anesthesia:**

- Analgesia - toe pinch, ear pinch
- Respiration - gross changes in rate, character of breathing
- Color of mucous membrane and skin - poor oxygenation (blue), poor blood perfusion (pale)
Procedures After Anesthesia and Surgery

After anesthesia and surgery, the following procedures are recommended.

Monitoring post anesthesia:

- Rabbits must be monitored until fully recovered from anesthesia as indicated by the ability to ambulate and maintain core body temperature.

Monitoring post procedure:

- Assess appearance, activity, and behavior as indications of pain and discomfort (see screen Detecting Pain and Distress).
- Assess food and water intake.
- Assess wound healing.
Euthanasia

The term euthanasia is derived from Greek and means "good death." Animals should be euthanized when killed for any purpose, including research. To euthanize a rabbit, you must be trained in the concepts of euthanasia, the method to be used, and the proper handling of rabbits.

Methods are classified as acceptable or conditionally acceptable, as set by the American Veterinary Medical Association's document, the AVMA Guidelines on Euthanasia. The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.

Methods

Click the link for each method below for recommendations on its use in euthanizing rabbits.

Acceptable Methods:

- Barbiturate overdose
- Inhalant anesthetic overdose
- Carbon dioxide (compressed tanks only)
- Carbon monoxide
- Potassium chloride in conjunction with general anesthesia

Barbiturates:

Excerpted from the AVMA Guidelines on Euthanasia –
Barbiturates
"Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia in ...[many species]. Intraperitoneal injection may be used... Intracardiac injection must only be used if the animal is heavily sedated, unconscious, or anesthetized."

Pentobarbital Combinations
"Several euthanasia products are formulated to include a barbituric acid derivative (usually sodium pentobarbital), with added local anesthetic agents or agents that metabolize to pentobarbital. Although some of these additives are slowly cardiotoxic, this pharmacologic effect is inconsequential. These combination products are listed by the DEA as Schedule III drugs, making them somewhat simpler to obtain, store, and administer than Schedule II drugs such as sodium pentobarbital. The pharmacologic properties and recommended use of combination products that combine sodium pentobarbital with lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives. A combination of pentobarbital with a neuromuscular blocking agent is not an acceptable euthanasia agent."

Conditionally Acceptable Methods:*

- Nitrogen
- Argon
- Cervical dislocation
- Decapitation
- Penetrating captive bolt

* The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.

Example Alternatives Search

For additional information on euthanasia of rabbits, including the impact of euthanasia agents on tissues, please refer to the database and websites below. Click on the database search terms to conduct your search online.

PUBMED search template:

- rabbits AND euthanasia

Websites:

- American Veterinary Medical Association, AVMA Guidelines on Euthanasia
- Working Party Report (Europe), Recommendations for Euthanasia of Experimental Animals
Diseases

Abortions
Adenocarcinoma of the Uterus
Back Injuries
Caked Udder
Cheyletiella parasitovorax Infestation
Coccidiosis
Cuterebra Warbles
Ear Mites
Enteritis
Mucoid enteropathy
Colibacillosis
Tyzzer’s Disease
Green Food Bloat
Fibroma
Fur-chewing Vice
Hair Balls
Heatstroke
Hutch Burn
Malocclusion (buck teeth)

Mastitis
Myxomatosis
Nest Box Mortality
Nosematosis
Pasteurellosis (snuffles)
Pregnancy Toxemia
Rabbit Fever
Rabbit Syphilis
Red Water (red urine)
Roundworms
Sarcoptic Mange
Sore Dewlap
Sore Hocks
Splay Leg
Subcutaneous Abscesses of Bunnies
Tapeworms
Trichophyton mentagrophytes
Vitamin E Deficiency

References

Federal Laws, Regulations, Policies:

5. USDA Animal and Plant Health Inspection Animal Care Policy Manual Policy #11 - Painful/Distressful Procedures
6. U.S. Government Principles For The Utilization And Care Of Vertebrate Animals Used In Testing, Research, And Training, Interagency Research Animal Committee

Guidelines:

Texts:

## GUINEA PIG - *Cavia porcellus*

<table>
<thead>
<tr>
<th>Trait</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>3 years average, 6-8 years maximum</td>
</tr>
<tr>
<td>Weight, Adult Male</td>
<td>1000-1200 grams</td>
</tr>
<tr>
<td>Weight, Adult Female</td>
<td>850-900 grams</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>100 grams</td>
</tr>
<tr>
<td>Breeding Age, Female</td>
<td>3-5 months, 500 grams</td>
</tr>
<tr>
<td>Breeding Age, Male</td>
<td>3-5 months, 550 grams</td>
</tr>
<tr>
<td>Estrus Cycle</td>
<td>16-19 days</td>
</tr>
<tr>
<td>Gestation</td>
<td>60-70 days, 63 average</td>
</tr>
<tr>
<td>Weaning age</td>
<td>10 days, 250 grams (born well-developed with fur, teeth, and eyes open; can run within hours of birth)</td>
</tr>
<tr>
<td>Begin Dry Food</td>
<td>4-5 days</td>
</tr>
<tr>
<td>Litter Size</td>
<td>2-5 piglets, 3-4 average</td>
</tr>
<tr>
<td>Time to Re-Breed</td>
<td>Spontaneous ovulators (10 hrs after estrus begins) cycle every 2-3 weeks</td>
</tr>
<tr>
<td>Breeding Life, Female</td>
<td>3-4 years</td>
</tr>
<tr>
<td>Breeding Life, Male</td>
<td>4-5 years</td>
</tr>
<tr>
<td>Mating</td>
<td>Pairs, Determined by vaginal plug, can palpate at 14-21 days</td>
</tr>
<tr>
<td>Chromosome Number</td>
<td>64</td>
</tr>
<tr>
<td>Body Temperature</td>
<td>101-104°F (average 101.5°F)</td>
</tr>
<tr>
<td>Respiration Rate</td>
<td>86 average, 80-150/minute</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>280 average, 240-400/minute</td>
</tr>
<tr>
<td>Blood Volume % Body Weight</td>
<td>65-90 ml/kg, 6-7%</td>
</tr>
<tr>
<td>Maximum Safe Bleed</td>
<td>7.7 ml/kg</td>
</tr>
<tr>
<td>RBC 1000/CU MM</td>
<td>5.2 average, 3-7</td>
</tr>
<tr>
<td>Hb G/100 ml</td>
<td>14.3 average, 11-17</td>
</tr>
<tr>
<td>PCV ML%</td>
<td>43.6 average, 37-50</td>
</tr>
<tr>
<td>Platelets 1000/CU MM</td>
<td>477 average, 250-750</td>
</tr>
<tr>
<td>WBC 1000/CU MM</td>
<td>11.2 average, 6-17</td>
</tr>
</tbody>
</table>
Sexing
Examine the distance between the guinea pig’s anus and its genitals (the vulva in females and the penis in males). The penis of the male guinea pig is located much further away from the anus than the vulva of the female guinea pig is. Look at the guinea pig pictures below. These are photographs of the anuses and genitalia of two individual guinea pig adults. The first guinea pig is a young male and the second cavy is a young female. I have blown the two images up to the same size (i.e. the guinea pigs in the photos are the same size) so that comparison of this anus-to-genitals difference in distance is easier to make.

Guinea pig pictures 1 and 2: The first photo is a picture of a male guinea pig’s genitalia and the second guinea pig photo is a picture of a female guinea pig’s genitals. Each
guinea pig has been positioned on its back to make guinea pig sexing easier to perform. Only keep guinea pigs restrained on their backs for a short period of time: just long enough for you to determine their sex. Keeping cavies on their backs for too long can be distressing to them because, being a prey animal species, they will feel like they are being held down by a predator and unable to get away.

What you will notice from these two images is that the distance between the anus and the penis of the young male guinea pig (sexing guinea pigs: image 1) is a significantly greater distance than the distance between the anus and the vulva of the young female guinea pig (sexing guinea pigs: image 2)

**Handling and Restraint Techniques**

Introduce your hand into the cage in a quiet, non-threatening manner. Gently grasp the pig over the back and shoulders, slipping your fingers under the front legs. As you lift the pig off the cage bottom, slide your other hand under its hindquarters to support its weight. Too vigorous a grasp over the thorax may injure the thoracic cavity and a pig may harm himself struggling if not adequately supported.

**Allergies**

Everyone working with laboratory animals should be enrolled in their institution’s occupational health and safety program. This program will help you monitor any development of allergies, since it is possible for people working with guinea pigs to develop allergies after some time.

It is essential that workers exposed to guinea pigs wear proper **personal protective equipment (PPE)**, including scrubs or disposable gowns, gloves, and shoe covers. If you are already allergic to guinea pigs, a respirator (such as an N95 type) can be worn to protect from airborne allergens. Persons who develop allergy symptoms should seek medical counseling from the occupational health and safety program. In severe cases, personnel may have to discontinue working with this species.
Zoonoses

In general, transmission of zoonotic disease from laboratory animals is uncommon because of ongoing efforts by both facility staff and vendors to monitor and improve the health status of animals using reliable health surveillance programs. Experimentally infected animals are a source of zoonotic transmission to humans. Health surveillance programs, routine sanitation, and personal protective equipment have important roles in preventing zoonoses.

Guinea pigs can be a reservoir of the following infectious agents which are transmissible to people:

Viruses:
- Lymphocytic choriomeningitis virus (LCM)

Bacteria
- *Salmonella* spp.

Fungi

Arthropods
- *Trixacarus caviae*

For more information, refer to *Occupational Health and Safety in the Care and Use of Research Animals*, published by the National Research Council.

Cage size requirements for guinea pigs are mandated by the Animal Welfare Act.

The Animal Welfare Act Regulations Section 3.28 specifies minimum cage sizes for guinea pigs. The interior cage height must be at least 7 inches (17.78 cm). Refer to the table below for minimum floor space for guinea pigs:

<table>
<thead>
<tr>
<th>Weight or stage of maturity</th>
<th>Minimum Floor Space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in²</td>
</tr>
<tr>
<td>Weaning to 350 grams</td>
<td>60</td>
</tr>
<tr>
<td>&gt;350 grams</td>
<td>101</td>
</tr>
<tr>
<td>Nursing females with their litters</td>
<td>101</td>
</tr>
</tbody>
</table>
For enrichment, guinea pigs may be provided shelter areas in their caging where they can hide.

**Acclimation**

Upon arrival to your facility, your guinea pigs should have an acclimation period before they are used in research studies. This period of time allows animals to adapt to a new environment. Effects of transportation stress include alterations in various blood parameters, immune cell function, food intake, and animal behavior. The period of time necessary for biological stabilization will depend on the parameters to be studied. Refer to your institution's attending veterinarian for recommendations that are appropriate for your project. Typically, acclimation periods range from 4 days to 1 week.

Example Alternatives Search:

For citations on the impact of transportation stress on guinea pigs, please click on the following search template to execute your search online:

PubMed

- guinea pigs AND transportation

**Quarantine**

Routine quarantine procedures may prolong the holding of your animals in special facilities.
Quarantine aims to prevent transmission of diseases between new animals and established colonies.

During quarantine, diagnostic tests are usually performed on the animals.

Acclimation and quarantine periods run concurrently, although they serve different purposes. Most institutions do not allow experiments on animals while quarantined.

**Assessing Pain and Distress**

If your proposed study involves a painful procedure, the animal use protocol application will ask for a method of assessing if the guinea pigs are experiencing pain or distress.

Assessing pain and distress in guinea pigs is difficult at times because guinea pigs are stoical animals, so that clinical signs of pain and distress may be subtle. Acute pain may cause vocalization, but guinea pigs in moderate pain often appear sleepy and are not likely to be aggressive. Whereas guinea pigs normally elude capture, a guinea pig in pain may be lethargic and easy to catch. The behavioral changes that reveal a guinea pig’s pain and distress may be detectable only with specialized behavioral methods.

**Clinical Signs**

Laboratory staff working with guinea pigs should be trained to recognize these abnormalities in:

- Activity level
  - Hypoactivity
- Behavior
  - Vocalization
  - Self-trauma
  - Ataxia
- Appearance
  - Cyanosis
  - Pale mucous membranes
• Vital Signs
  o Respiratory distress
• Body Condition
  o Weight loss
  o Emaciation
  o Dehydration.
• Intake
  o Reduced intake of food and water

The guinea pig shown below has swollen feet (note the front foot especially), inflamed skin, hair loss, and skin encrustations. This animal is in severe pain and/or distress.

Chronic Pain and Distress

A chronic state of pain or distress may be more subtle and difficult to detect. A good knowledge of the animal’s normal appearance and behavior is especially important to recognize chronic pain or distress.

For methods on assessing and alleviating pain and distress in rodents, refer to the course: Post Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress. The concepts described are applicable to guinea pigs, and the methods may be adapted to this species.

Genetics

Inbred strains and outbred stocks of guinea pigs produce animals that are used for different purposes. The decision to use isogenic inbred strains or non-isogenic outbred stocks is determined by the experimental strategy.

• Commonly used inbred strains include Strain 2/Nsim and Strain 13/N. Inbred strains are used for genetic engineering and finely controlled studies that capitalize on genetic isogenicity. Inbred strains with characteristics of human diseases or physiological conditions are generally preferred models for biomedical research.
• Commonly used outbred strains include:
  o Dunkin-Hartley (albino) - nomenclature: HsdPoc:DH
  o Hartley (albino) - nomenclature: Crl:(HA)BR
  o Pigmented breeds are available.
• In addition, a hairless, euthymic mutant strain is also available.
  o IAF (hairless, euthymic, albino) - nomenclature: Crl:IAF(HA)-hrBR

Please check with your animal resource department for information on vendor choices as animal source affects animal health status.

**Biological Features**

![Image of guinea pigs]

Though guinea pigs share many anatomical and physiological features with humans, guinea pigs have many unique biological characteristics. Knowledge of species-specific characteristics is helpful to effectively manage these animals and to plan experimental procedures for their use. Researchers should be aware of the practical features of guinea pig anatomy and biology.

**Handling**

Guinea pigs are non-aggressive animals that seldom, if ever, bite. Use two hands to pick up an adult guinea pig; grasp it gently around the thorax and support the hind end.

**Startle response**

When guinea pigs are surprised or frightened, they may either freeze or stampede about the cage. When they stampede, guinea pigs may leap out of a cage with low walls, i.e., less than 10 inches (25 cm). If young are present, they can be injured by stampeding adults.
Gastrointestinal

Teeth

All teeth in the guinea pig are open-rooted, meaning that the teeth grow continuously throughout adult life. Continuous tooth wear is necessary to allow the guinea pig to take in and consume food. Tooth malocclusion and overgrowth occurs due to congenital anomalies (mandibular) and/or dietary insufficiencies (e.g., intermittent hypovitaminosis C).

The premolar teeth are most likely to be affected. Premolar overgrowth is difficult to detect because of the guinea pig’s narrow mouth and the large mucosal folds concealing the teeth. The tongue and cheek can be abraded and lacerated by the overgrown teeth.

Affected animals may have wet fur around the mouth due to salivation (slobbering). Guinea pigs with maloccluded and overgrown teeth exhibit a decrease in food consumption and body weight. Staff must be alert to detect any signs of this condition and to provide appropriate treatment.

Inability to vomit

Guinea pigs do not vomit because they lack the neurophysiological mechanisms for emesis. Therefore, presurgical fasting is not necessary in guinea pigs, as it is for nonrodent species. However, if endotracheal intubation will be performed, overnight fasting is essential to clear the mouth and laryngopharynx of food material. If animals are not fasted overnight, the endotracheal tube will become contaminated with food particles en route to the larynx, and food will be introduced into the trachea. Food contamination of the respiratory passages will cause inflammation of the respiratory tract lining and possibly pneumonitis.

Dietary requirements

Like primates, guinea pigs are unable to synthesize vitamin C (ascorbic acid) and have a high dietary requirement: 5 mg/kg vitamin C per day for maintenance and 30 mg/kg vitamin C per day during pregnancy. Mainly because of this requirement, guinea pigs should only be fed diets prepared specifically for guinea pigs (vitamin C enriched). Foods must be used fresh since vitamin C degrades rapidly. Pelleted diets must be fed within the period specified by the manufacturer, the shelf life (90 or 180 days depending on the product).

Drinking water can be fortified with vitamin C for an alternative source, but must be prepared daily.

Aside from their high vitamin C requirement, guinea pigs should not be fed diets for other laboratory species. Besides being deficient in vitamin C, rabbit diet, for example, has too much vitamin D and too little folic acid for guinea pigs.
**Feeding habits**

Guinea pigs develop rigid habits for food item choices, and watering and feeding systems. Within a few days of birth, they become imprinted on types of acceptable foods and types of feeder and watering systems (e.g., crock, sipper tube). Later, guinea pigs may have difficulty adapting to changes in these items. They may stubbornly resist change despite dehydration and starvation.

Guinea pigs are notorious for playing with water bottle sipper tubes causing the cages to flood. When possible, sipper tubes may be mounted so that just the tip protrudes into the cage thus reducing the possibility that the guinea pigs can manipulate it and cause it to leak.

Water sipper tubes often become clogged by guinea pigs. Guinea pigs often have food in their mouth while drinking, and food materials may plug the sipper tube. It’s important that husbandry staff check the operation of sipper tubes daily.

**Coprophagy**

In guinea pigs, herbaceous foodstuffs are broken down by microbial action in the cecum, which is a large organ in the guinea pig. To assimilate the microbial byproducts of digestion, the guinea pig regularly eats its own feces, a habit known as coprophagy. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the guinea pig.

**Sensitivity to antibiotics**

Antibiotics, especially those with a Gram-positive spectrum of activity, like the penicillin family, may cause a toxic shock syndrome in guinea pigs. Antibiotic that reaches the intestine, e.g., by biliary excretion, kills some of the intestinal flora and allows an overgrowth of resistant Clostridium species. With proliferation of these species, clostridial toxin is produced and released. The animal develops a hemorrhagic cecitis and colitis. The guinea pig may present with diarrhea initially and die very quickly.

**Unique Immune System**

**Kurloff cells**

Kurloff cells are T-lymphocytes (Natural Killer effector cells) that have a distinct microscopic appearance unique to the guinea pig. Kurloff cells contain acidophilic intracytoplasmic inclusions. These cells are found in highest numbers in the placenta.
Complement

Mature guinea pig females are used as a source of serum complement.

Infectious

- *Bordetella bronchiseptica* contagion

Guinea pigs are susceptible to *Bordetella bronchiseptica*, which is often harbored by other common laboratory species, such as rabbits (not specific pathogen free or non-SPF), dogs, cats, swine, birds, and nonhuman primates. Guinea pigs should not have contact with these species or contaminated fomites. Infected guinea pigs may develop pneumonia and upper respiratory tract disease.

Metabolic

- High rate of metabolism – impact on drug clearance

The guinea pig's high rate of metabolism produces a rapid clearance of drugs from the body. Drugs administered at dose rates used in larger species (with lower metabolic rates) would reach lower blood concentrations and exert less pharmacological effect in the guinea pig. As a result, guinea pigs should receive drug doses that have been scaled to the guinea pig's metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

In general, guinea pig-specific dose rates have been determined and are widely published for drugs that are commonly used in animal research, such as anesthetics, analgesics, sedatives, and antibiotics. Investigators are advised to obtain guinea pig dose rates from laboratory animal references or from their institution's veterinary staff.

Reproduction

- Dystocia in late-bred sows
- Precocious young

**Dystocia**

A female to be bred must be mated by 6 months of age to prevent dystocia subsequent to incomplete relaxation of the pubic symphysis at parturition. Under the effect of relaxing, the pubic symphysis begins to separate in mid-gestation, and the gap continues to widen gradually until parturition. Symphyysis separation is necessary for vaginal delivery of the young. If breeding is delayed until the sow is 7 or 8 months of age, the symphysis does not separate to the same degree and dystocia may result, especially if the neonates are large. In such a case, cesarean section would be necessary.
**Precocious young**

Guinea pigs are precocial, i.e., they are born fully furred, eyes and ears open, teeth erupted, and almost self-sufficient. The young do best when they can be nursed and so should stay with their dams for 2-3 weeks. However, they can be hand-reared if necessary. Hand-reared neonates require softened food. They also require, for about one week, a heat source and regular tactile stimulation for urination and defecation. Nursing mothers will readily foster young from another litter, but the amount of milk may be limiting for a large number of neonates. Fostering is used for generating specific pathogen-free colonies.

Respiratory

- Endotracheal intubation
- Bronchoconstrictor sensitivity

**Endotracheal intubation**

Guinea pigs regularly retain food material in the oropharynx and laryngopharynx. If endotracheal intubation is to be performed, overnight fasting is essential to clear these areas of food material. If animals are not fasted overnight, the endotracheal tube will become contaminated with food particles en route to the larynx, and food will be introduced into the trachea. Food contamination of the respiratory passages will cause inflammation of the respiratory tract lining and possibly pneumonitis.

**Bronchoconstrictor sensitivity**

Administration or massive release of histamine causes a severe, often lethal, bronchoconstriction in the guinea pig.

Miscellaneous

- Foot injury

Guinea pigs should not be housed on wire mesh floors. Being larger than other laboratory rodents, guinea pigs have a risk for a traumatic condition of the feet, known as pododermatitis, when they are housed on mesh flooring. Pads of the affected feet are typically swollen, reddened, and ulcerated.

If guinea pigs raised on solid flooring are transferred to a mesh flooring, they are also prone to leg fractures. This may occur if their feet slip and fall through the mesh holes.
Injections and Blood Collection

Volume Recommendations

Below are volume recommendations (ml) for acute intravenous fluid administration and blood collection in adult guinea pigs based on the references provided below. These recommendations are currently in the process of being reviewed and updated; if you have any questions regarding correct volumes, please consult your facility veterinarian.

<table>
<thead>
<tr>
<th>Sex</th>
<th>IV Fluid Volume (ml)</th>
<th>Tot. Blood Volume (ml)(^b)</th>
<th>Safe Bleed Volume (ml)(^c)</th>
<th>Bleed-out Volume (ml)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0.5</td>
<td>63-90</td>
<td>6-9</td>
<td>29-42</td>
</tr>
<tr>
<td>F</td>
<td>0.5</td>
<td>52-67</td>
<td>5-7</td>
<td>24-31</td>
</tr>
</tbody>
</table>

\(^a\)Males 850 – 1200 g; Females 700 – 900 g.

\(^b\)Mean total blood volume 75 ml/kg; mean total plasma volume 39 ml/kg.

\(^c\)Maximum safe bleeding volume 7.7 ml/kg. Removing greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids to replace the volume of blood collected. In addition, monitor the animal's hematocrit for anemia.

\(^d\)Animals should be exsanguinated only under anesthesia.

From:

Peripheral Blood Vessels

Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 25 to 27 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Vessels</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Dorsal metatarsal vein   | 1. Accessing these veins:  
  o Does not require anesthesia  
  o May be aided by sedation because vein visibility is enhanced by peripheral vasodilation (drug effect)  
  o May be aided by sedation to reduce animal struggling due to distress  
  2. Small amounts of blood may be obtained from the ear vein by:  
    o Lacerating the vein and collecting blood in a capillary tube  
    o Cannulating with a fine needle (e.g., 29 ga) |
| Lateral saphenous vein   |                                                                                                                                                                                                       |
| Medial saphenous vein    |                                                                                                                                                                                                       |
| Ear vein                 |                                                                                                                                                                                                       |
| Jugular vein             | Venipuncture by these routes is commonly performed under anesthesia because of the need for restraint or relief of pain or distress.                                                                |
| Penile vein (males only) |                                                                                                                                                                                                       |
| Toenail clipping         |                                                                                                                                                                                                       |
| Cardiac puncture         | 1. These two methods above require anesthesia.  
  2. Both methods are most often allowed only as a terminal procedure because of the risk of serious injury to the animal.  
  3. Check with your institution for guidelines on these routes of blood collection.                                                                 |
| Orbital venous sinus     |                                                                                                                                                                                                       |
Nonvascular Injections

Below are the nonvascular routes of injection that are commonly used in guinea pigs. Included are volume recommendations for the safe administration of fluids acutely in adults (weight range 700 – 1200 g). Recommended needle sizes for parenteral injections are 23 to 25 gauge; larger needles may be necessary for injecting viscous materials.

Subcutaneous (SQ or SC)
5-10 ml (scruff of neck)
1-2 ml (flank)

Intraperitoneal
(IP) - 10-15 ml

Intradermal
(ID) - 0.1 ml/site

Intramuscular
(IM) - 0.3 ml per site

Oral
(PO) - 5 ml (gavage)

From:

Surgery

Surgery on guinea pigs should be performed in a location dedicated to surgery and related activities when used for that purpose. This is best achieved by using an animal procedure room or surgery suite within the animal facility. The location used for the operative field should be cleaned and sanitized before use. Materials and supplies used in support of the procedure should be positioned and managed to avoid contaminating sterile areas.

Aseptic Technique

Aseptic technique should be used when performing surgery on guinea pigs. This is required by the Animal Welfare Regulations. The standards described here are consistent with the Guide for the Care and Use of Laboratory Animals. Nonaseptic methods are not acceptable. In addition, guinea pigs have a low tolerance to many antibiotics. Antibiotics that allow overgrowth of Clostridial species cause a rapidly fatal condition of acute enteropathy and toxic shock in guinea pigs. Therefore, aseptic technique must be rigorously applied in guinea pig surgery to prevent infection.
Supportive Care and Monitoring

Supportive care aims to:

- Maintain the animal’s physiological status as near normal as possible
- Minimize animal pain and distress

Supportive care includes the monitoring of both physiological parameters and analgesia during anesthetic and surgical procedures. Monitoring of vital signs and pain should be conducted throughout the procedure and the recovery period.

Surgical and Anesthesia Problems

Keep in mind that:

- General anesthesia causes disturbances of thermoregulation and other physiological functions. Some animals are unable to properly thermoregulate for some time after some procedures, including anesthesia and surgery. Maintaining body temperature, e.g., via insulating materials and heating pads, is an important objective of supportive care.
- During surgery, the animal may experience pain if anesthesia is inadequate at any time during the procedure.
- Postoperatively, the animal may experience pain, discomfort, and distress unless treated with analgesics and appropriate supportive care measures.
Due to the interaction of metabolic factors and drug effects that can cause animal mortality, guinea pigs should receive good supportive care and monitoring during anesthesia, whether or not the procedure involves surgery.

Supportive Care and Monitoring: Procedures

During anesthesia and surgery, the following procedures are recommended.

Supportive care:

- Provide a source of warmth to guinea pigs from the onset of anesthesia to the end of anesthetic recovery.
- Inject sterile physiological fluid (warmed) to compensate for blood loss during a procedure and depressed fluid intake post-procedure.

Monitoring during anesthesia:

- Analgesia – toe pinch, ear pinch.
- Respiration – gross changes in rate, character of breathing.
- Color of mucous membrane and skin – blue (poor oxygenation), pale (poor blood perfusion).

Postsurgical Care

After anesthesia and surgery, the following procedures are recommended.

Monitoring post anesthesia:
• Guinea pigs must be monitored until fully recovered from anesthesia as indicated by the ability to ambulate and maintain core body temperature.

Monitoring post procedure:

• Assess appearance, activity, and behavior as indications of pain and discomfort ("Detecting Pain and Distress" lesson).
• Assess food and water intake.
• Assess wound repair.

Euthanasia

The term euthanasia is derived from Greek and means "good death." Animals should be euthanized when killed for any purpose, including research. To euthanize a guinea pig, you must be trained in the concepts of euthanasia, the method to be used, and the proper handling of guinea pigs.

Methods are classified as acceptable or conditionally acceptable, as set by the American Veterinary Medical Association’s document, the AVMA Guidelines on Euthanasia. The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.
Methods

Click the link for each method below for recommendations on its use in guinea pigs.

Acceptable Methods:

- Barbiturate overdose
- Inhalant anesthetic overdose
- Carbon dioxide (compressed tanks only)
- Carbon monoxide
- Potassium chloride in conjunction with general anesthesia

Carbon Dioxide

Reformatted from the AVMA Guidelines on Euthanasia –

"Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely.

"Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (eg, antacids) is unacceptable.

"Species should be separated and chambers should not be overcrowded.

"With an animal in the chamber, an optimal flow rate should displace at least 20% of the chamber volume per minute. Loss of consciousness may be induced more rapidly by exposing animals to a CO₂ concentration of 70% or more by pre-filling the chamber for species in which this has not been shown to cause distress.

"Gas flow should be maintained for at least 1 minute after apparent clinical death. It is important to verify that an animal is dead before removing it from the chamber. If an animal is not dead, CO₂ narcosis must be followed with another method of euthanasia. Adding O₂ to the CO₂ may or may not preclude signs of distress. Additional O₂ will, however, prolong time to death and may complicate determination of consciousness.

"There appears to be no advantage to combining O₂ with carbon dioxide for euthanasia."

SQ- larger volumes (5 – 10 ml) given into “space” created by tenting skin over nape of neck or back
IP- Invert animal by gently grasping animal over the thorax and hindquarters, presenting the animal's abdomen to the injector with the head tilted slightly toward the floor. Insure that a needle of sufficient length is utilized to enter the peritoneal cavity (i.e., 1”); enter the needle off the midline approximately 1/3 the distance from the pubis to the umbilicus. Watch for aspirate; discard contaminated solutions.
IV- saphenous veins – suggest 24 or 25g needle
### Diseases

<table>
<thead>
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<td>Bacterial Pneumonia &amp; Septicemia</td>
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<tr>
<td>Balantidiasis</td>
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<td>Pinworms</td>
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<td>Salivary Gland Virus</td>
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<tr>
<td>Salmonellosis</td>
<td>Scrotal Plug</td>
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<tr>
<td>Scurvy</td>
<td>Slobbers</td>
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<tr>
<td>Soft Tissue Calcification</td>
<td>Staphylococcal Pododermatitis</td>
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<tr>
<td>Typhlocolitis</td>
<td>Vaginitis</td>
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<tr>
<td>Yersinia</td>
<td></td>
</tr>
</tbody>
</table>

### References

#### Federal Laws, Regulations, Policies:

1. Animal Welfare Act, as Amended (7 USC, 2131-2156)
6. **U.S. Government Principles For The Utilization And Care Of Vertebrate Animals Used In Testing, Research, And Training, Interagency Research Animal Committee**.

#### Guidelines:


#### Texts:

IX. **Minimizing Pain and Distress**

Investigators are responsible for minimizing pain and distress in research animals by:

- Judicious use of anesthetics and analgesics
- Refinement of experimental techniques
- Implementation of best practices
- Implementation of humane endpoints

Two critical components in the refinement of experimental techniques are:

- Monitoring animals for pain and distress, and
- Using interventions for reducing pain and distress.

Federal animal welfare laws, regulations, and policies mandate the scientist’s responsibility for the humane care and use of animals in research. A concise description of the requirements for the humane care and use of laboratory animals is given here in the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.*

Principles IV – VIII, U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, from the Public Health Service Policy on Humane Care and Use of Laboratory Animals:

- IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

- VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

- VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

Minimizing Sources of Nonexperimental Variation
Supporting the Integrity of Research Data

Maximizing the humane care and use of laboratory animals and minimizing confounders of experimental variation are mutually complementary objectives of research animal management. Both support the integrity of the research data. Achieving humaneness in animal research depends upon the control, and whenever possible, the reduction of animal pain and distress. Minimizing pain and distress also reduces the impact of these extraneous factors on the research, i.e., as sources of non-experimental variation.

Example:

In a mouse model of experimental autoimmune encephalomyelitis, implementation of supportive treatment (hydration and nutrition) was shown to protect against loss of body weight and to greatly extend survival of animals on study, from 25 to 60 days. (Ref.: Lab Animal, 29(5): 40-46, 2000.)

Systematically Monitoring for Pain and Distress

A best approach to minimizing animal pain or distress is to systematically monitor animals after a procedure or when illness is expected.

How often the animals should be monitored depends on the:

- severity of the animals’ condition,
- expected rate of change in the animals’ status, and
- impact of the procedure on the animals.

At a minimum, all animals should be evaluated once daily.

However, the nature of the procedure and condition of an animal may dictate that the animal be assessed multiple times a day. As mentioned on the previous screen, smaller mammals may experience physiologic changes such as chilling and starvation faster than larger animals. Therefore, rodents may require more frequent monitoring than larger animals. Some situations may require hourly or even continuous monitoring during critical periods in which rapid change in an animal’s condition would be anticipated.

This course offers a systematic daily approach for assessing clinical signs of rodent pain and distress. Some clinical signs may require assessment at a greater frequency to focus on parameters of particular relevance to the specific model and to provide the animals with appropriate intervention to minimize pain/distress.
Detecting Clinical Signs of Pain and Distress

The image shows rats with sleek hair coats that are moving around their cage. Normal feces are present in the bedding. The rats appear relatively normal from this top view. However, the rats in the far left upper corner and the rat in the lower left corner should be checked a little more carefully as they are hidden and perhaps may be head-pressing, which is a sign of distress.

Signs of pain and distress in rodents are not easy to detect because of their:

- small body size
- tendency to conceal outward signs of pain and distress, and
- habit of hiding or freezing when disturbed.

Nevertheless, signs of pain or distress can be detected in rodents by carefully observing subtle changes in behavior. The ability to properly assess pain and distress in rodents requires:

- knowledge of normal rodent behavior and appearance
- systematic approach to observing clinical signs in rodents

Appearance and Behavior: Observations

The first step in assessing clinical signs of pain and distress is a gross inspection of rats or mice for abnormalities in appearance and behavior in their home cage. This assessment takes only a few minutes for the practiced observer.

Note that very young pups should be observed very carefully in order to avoid upsetting the mother and causing pup rejection.

1. From the Cage Exterior

Routinely inspect the rodents through the top and sides of the cage. Get in the habit of removing the cage from the shelf and looking through all sides of the cage. Signs of distress

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may be missed in animals on lower or upper shelves because of low lighting or difficult access. Baby mice and rats can be inconspicuous within piles of bedding or nestboxes.

Photo:

These rats below are not having problems after surgery. They are sleeping the way one would expect and they appear comfortable. They are clean, have normal hair coats, good color (skin and mucosa), and normal vital signs.
Physical Exam for Clinical Condition

In the image, the rats appear distressed. The investigators on this study believed that this was normal for day one postoperatively because the animals were moving. However, one can see head-pressing, no evidence of grooming, and porphyrin staining in these rats. One rat (bottom) does not move his tail in a normal way. A physical exam of this animal revealed low body temperature, hind limb weakness, anemia, pain, and weight loss.

After assessing the animals’ appearance and behavior (preceding screen), conduct a physical exam using methods that are appropriate to the species and experimental model.

Performing a clinical exam on rodents is somewhat limited compared to larger animals due to the greater difficulty in venous access and the smaller sampling size of biological fluids. Nevertheless, specific methods and equipment for rodents allow a clinical exam to provide information on animal well-being.

In the image, the rats appear distressed. The investigators on this study believed that this was normal for day one postoperatively because the animals were moving. However, one can see head-pressing, no evidence of grooming, and porphyrin staining in these rats. One rat (bottom) does not move his tail in a normal way. A physical exam of this animal revealed low body temperature, hind limb weakness, anemia, pain, and weight loss.

Quantifiable Characteristics

In conducting a physical exam, use quantifiable characteristics whenever possible. These can be tracked over time and compared to a starting baseline or to normal, untreated animals. Such measurements are not only helpful for clinical assessments, but they can also be useful when compiling research data and writing manuscripts. Later in this course, simple record-keeping methods will be discussed to help utilize this information.

You may evaluate:

- Behavior
• Body weight
• Surface lesions (wounds, masses)
• Hydration status
• Body temperature (telemetric methods)
• Blood parameters (Blood collection can be difficult/stressful in mice; may be used to confirm disease or failed treatment.)

Specific Examination Procedures

Specific physical exams may be added to list on the preceding screen to facilitate the detection and monitoring of illness, pain, and distress that result from your study procedures.

For example:

• A neuromuscular exam can be conducted with simple techniques to measure hindlimb or forelimb strength and neurological deficits.

• Abdominal palpation (gentle) of the abdomen may detect pain due to peritonitis. (In rats, listen for vocalization or grunting or breath-holding by placing the animal close to your ear.)

Later screens describe a systematic approach for a typical physical exam. Methods to treat abnormalities are included in this discussion.

Body Weight: Assessment

Measuring body weight is a quick way to determine whether an animal is eating and drinking. Body weight changes are a sensitive indicator of rodent health, and a baseline weight measurement allows monitoring of the experiment's impact on the animal. Reduction in body weight may reflect starvation, dehydration, or a combination of both.

Failure of young animals to gain weight is equivalent to a loss of body weight. Therefore, body weight changes should be interpreted in terms of both actual loss of weight and lack of expected growth. It is helpful to compare body weights of treated animals with those of normal controls.
The body weights of mice and rats can vary dramatically depending on stock or strain. Refer to the weight curves on each strain or stock available from the animal vendor.

In addition to measuring body weight, you should assess body condition. (This was briefly mentioned in Lesson 6, Appearance and Behavior: Assessment.) Rodents can be assessed for emaciation or cachexia (body wasting) by examining and palpating the lumbar spine and iliosacral areas. A scoring system can be applied to the progressive loss of fat and muscle mass to gauge the severity of emaciation.

Approaches for nutritional supplementation will be described in this lesson. For treatment of hydration, refer to Lesson 9.

**Body Condition Assessment and Scoring**

Mice and rats can also be assessed for body condition which is one indicator of health status. For some situations, such as tumor studies, it may be more accurate to assess an animal’s body condition, rather than its overall weight.

Endpoints or other intervention criteria may be based on a score of body condition. Scales have been developed to standardize this process.

The graphic below is taken from Figure 1. of the journal article:
Example

Photo:

In the image below, the mice are huddled. The mouse on the left has piloerection and a poor body condition. This animal has a generalized loss of muscle mass, making the spine prominent. One can palpate along a mouse’s back and pelvic area to determine the extent of loss in the muscle mass.
Nutritional Support

Animals recovering from surgery develop a negative nitrogen balance as do human surgical patients. Young rodents are especially vulnerable to starvation because they lack long term fat and glycogen stores. Rodents typically have a reduced food (and water) intake 1-2 days after surgery. Low food intake may be more severe and more prolonged if animals are experiencing pain and distress (e.g., if pain alleviation is inadequate).

Returning animals to a physiological plane that is as close to normal as possible is nearly always consistent with the scientific objectives of the study. Thus, the impact of surgery on the experimental model should be minimized. Nutritional support (as well as fluid and electrolyte therapy) is important for enhancing an animal’s recovery after surgery.

Nutritional support can also be important for nonsurgical studies in which morbidity and reduced food intake occurs. If you have included weight loss as a humane endpoint, you can actually generate false negative findings simply by failing to provide adequate nutritional support during the peak impact of a study. This is detrimental in research on interventions designed to help animals overcome sickness.

Some examples of nutritional support include:

- peanut butter
- fresh fruit
- baby rice cereal
- high protein or high fat drink (detailed on next page)

High-Protein and High-Fat Diet

Stimulating appetite to increase food intake is helpful to promote a more rapid recovery in rodents as in other species. Something that tastes different and better than the normal everyday diet may be appealing to rats and mice and so may stimulate their appetite. Although some studies may have restricted nutrient requirements, the provision of a homemade or sterile commercially prepared supplement can be helpful to increase food intake and to maintain homeostatic controls such as caloric intake, electrolyte balance, and insulin/glucagon ratio.
Commercial diets formulated specifically for rodents recovering from a surgical procedure may be used for balanced nutrition and fluid source, e.g., Surgical Transgel® (Charles River Laboratories). In addition, peanut butter has been used to tempt rodents to eat.

A high-protein and high-fat diet, which may coax an inappetant rodent to eat, can be prepared as follows:

1 cup hot water
1 package raspberry Jell-O
30 ml STAT VME High Calorie Liquid® (by PRN)
20 ml Pediasure® (by Abbott Laboratories)
2 scoops Designer Protein™ (by Next Proteins International)

Blend well.
Pour into ice cube trays.
Refrigerate.

Feed the above diet at a rate of:

- 1/4 cube per rat per day
- 1 cube per cage of mice (5) per day

**Fluid and Electrolyte Balance**

Maintaining normal homeostasis is greatly dependent on osmotic pressure between tissue spaces. Fluid and/or electrolyte imbalance resulting in dehydration or edema may produce discomfort and add to pain and distress resulting from other causes. Also, animals in pain and distress are likely to have reduced fluid and food intake and so may develop dehydration secondarily. Rodents commonly become dehydrated due to experimental procedures that affect their water intake. Therefore, scientists and caregivers must be able to assess and control hydration.

Performing the exam:

- Observe the animals' behavior. Rodents that are dehydrated may be sluggish.
- Assess the animals' appearance.

    Useful indices of hydration:

    - Skin turgor:

        To assess skin turgor, tent the skin. Grasp, lift, and twist a fold of skin over an animal's back and watch the skin fall downward into normal position. Compare the response in a normal animal. In a dehydrated animal, the skin is less elastic and may remain tented longer and return more slowly to normal position.

    - Hair coat

    - Eye clarity
- Shape and position of the eye within the orbit
  - Blood may be collected (in rats) for measuring total serum protein and electrolytes.

**Over-hydration**

In conditions of diuresis and low specific gravity, urine may be collected for measuring urine specific gravity on a refractometer.

Since rodents often urinate when picked up, you can be ready with a tube to collect a sample. You may also gently express the bladder. To locate the bladder, gently palpate the caudomedial abdomen while the animal is hand-restrained. The bladder will feel like a pea-sized structure. Be careful to avoid traumatizing the bladder! Excessive force will cause the bladder wall to hemorrhage, and blood will appear in the urine.

A clinical refractometer is an inexpensive hand-held device that measures specific gravity and total protein. Rodent urine typically has a high specific gravity and so a small animal instrument should be used rather than one designed for humans. Although here, too, rodent urine specific gravity is likely to be above the scale. Therefore, the use of a refractometer will be more useful in conditions associated with diuresis and low specific gravity.

Commercial urine dip sticks also measure urine specific gravity as well as urine creatinine, blood, leukocytes, protein, ketones, pH and bilirubin.

**Treatment of Imbalance of Fluids and Electrolytes**

Rodent discomfort and morbidity can be minimized with:

- Adequate administration of fluids
- Monitoring for clinical dehydration

Providing supplemental fluids during experimental studies where there is predictable morbidity is often helpful for optimizing well-being in rodents.

There are two common approaches for maintaining hydration status:

1. Administering fluids proactively without assessing hydration status, based on the assumption that most animals in the study will have a similar degree of dehydration.
2. Assessing hydration status and then formulating a fluid dosage to normalize hydration. This approach customizes the treatment for each animal and avoids over-hydration.

**Treatment, continued**

Normal maintenance volumes of Lactated Ringers Solution, or 0.9% saline, or glucose-saline can be injected in amounts of 1-2 ml/25 g mouse and about 5-10 ml/250 g rat per day. The subcutaneous administration of these volumes may begin prior to a study and continue once daily (or split in two doses a day) throughout the period of expected morbidity.
Therapeutic fluids should be warmed prior to injection because fluids administered at room temperature will chill the animal. Fluids can be loaded into syringes and kept warm in rodent support areas.

Analgesic treatments may be combined with daily fluid administrations (for hydration therapy). For convenience in treating multiple animals, you can figure the total fluid volume needed for the study and add the appropriate amount of analgesic to a concentration that will deliver the desired dose in each aliquot administered.

For more information on medications, refer to Lesson 12, Alleviation of Pain and Distress: Pharmacological Treatment.

**Body Temperature: Assessment**

Due to their large ratio of body surface area to mass and high metabolic rate, rodents lose body warmth at a faster rate than do larger animals. Conventional thermometers are not practical for use in rodents and can cause stress if used in unanesthetized rodents.

In studies of toxicology, sepsis, diabetes, or whenever morbidity is expected to be high, investigators may consider the use of implantable microchips to track body temperature (as well as to identify an animal) without the need for animal manipulation. Microchips can be injected under the skin using conventional restraint or light inhalation anesthesia. Check with your institution’s veterinary staff for information on purchasing a microchip system. (The noise of the microchip reader can frighten a rodent. Consider placing the chip in the animal's rump as opposed to the neck.)

Body temperature is also a useful adjunct in the monitoring of humane endpoints in rodents because a reduction in temperature of sufficient magnitude can be a reliable predictor of death. Body temperature measurements may guide the decision of when to euthanize an animal, which will end or prevent unnecessary pain/distress and allow for the antemortem harvest of fresh body tissues for histopathologic or other analysis.

**Hypothermia**

When under general anesthesia, rodents lose heat very rapidly. A mouse can lose 1 degree of body temperature per 5 minutes. A best practice is to use methods for conserving body heat during a procedure that will induce hypothermia, such as anesthesia and surgery. These methods are the provision of a heat source, thermal insulation, or a combination.

Caution! Warming devices should provide gentle heat only (maximum of 40 °C or 104 °F). Having a high ratio of body surface area to mass, rodents on a heat source heat up as quickly as they lose body heat when chilled. They can readily overheat when high temperature heating systems are used, causing animal injury or death.

Photo:

The image below shows a rat with burns of the ears from over-utilization of a heat lamp. Burns can occur when a heat lamp is positioned too close to the animal. Heat lamps are generally discouraged because it is difficult for animals to escape the heat when they are too hot.
Treatment of Hypothermia

There are many practical ways to provide temperature support to rodents, either individually or in cages. Select the following links for examples of practical approaches for conserving body warmth in rodents during an experimental procedure:

- Insulated pouch or wrap
- Insulated pads
- Using warming pads
- Chemical warming pads (often too hot)
- Circulating water warming pads
- Electrical heating pads (use of human-grade pads is strongly discouraged)
- Warming racks
- Heat lamps (use is strongly discouraged)
- Monitoring area temperature

For animals recovering from anesthesia, body temperature may remain low beyond the time the animals begin to ambulate. Therefore, it is best to keep them warm until their activity has returned to normal. In addition, if recovering animals are warmed within a cage, offer an area for escape from the heating device. This will allow recovering animals to leave the heated area for a cooler part of the cage if they become too hot.

Tumors: Pain and Distress

The growth of solid or ascitic tumors produces pain and distress in rodents just as in humans and other animals.

Examples:

- Pain is associated with distension of overlaying tissues and ulceration of involved skin.
- Tumors that impinge on joints can impair body movement and locomotion and can restrict the animal’s access to food and water.
• Growth of a tumor (any type) may cause the animal not to eat and lose body condition.

**Tumors: Assessment**

Develop an approach that evaluates both the general effects of cancer, e.g., inappetance, and the specific problems related to the type and placement of the tumor.

Assessment of the clinical condition of a tumor-bearing rodent largely depends on characteristics of the tumor’s biology, such as:

• tumor growth rate
• invasion
• distension
• ulceration
• metastasis
• production of cachectic factors

The body systems most likely affected by the tumor should be identified and examined for clinical signs of illness. Therefore, the tumor model will determine the clinical signs to be monitored.

Examples:

• Superficial tumors - ulceration, swellings
• Intracranial tumors - neurological signs
• Ascitic tumors - abdominal distension, dyspnea

Although clinical signs may be anticipated, as related to the tumor biology and location, be mindful that unexpected signs may also occur.

**Tumors: Endpoints**

Unless otherwise approved by the IACUC, animals should be euthanized before they become moribund or die due to tumor load. Also, animals should be euthanized before the tumor mass becomes excessive, ulcerates, or impairs the animal’s bodily functions or behavior.

The criteria for endpoints in tumor development should be established in the animal protocol. These are generally a combination of:

• Tumor mass or burden (many institutions have specific tumor size guidelines)
• Body condition, e.g., cachexia (emaciation)
• Impairment of body functions, e.g., gait
• Ulceration
Unless other arrangements have been made, it is usually the investigators’ responsibility to euthanize animals that have reached their endpoints. Investigators should monitor their animals often enough so that endpoints are never passed. Allowing animals to go past their endpoints can be considered a protocol violation and may be acted upon by the IACUC if it occurs with any frequency.

**Example**

Photo:

The image below shows a nude mouse with an implanted tumor. The mouse has reached a humane endpoint in the experiment because of the tumor’s size and because the tumor has become necrotic and ulcerated. This mouse was euthanized.

![Mouse with tumor](image1.jpg)

**Alleviation of Pain and Distress**

The detection and alleviation of pain or discomfort in rats and mice have been discussed in this course. The effective recognition of pain and distress should not rely on a single clinical observation but rather on a composite of signs and measurements that together reflect animal well-being in terms of pain or distress.

Photo:

In the image, a rat is shown 36 hours after a neurosurgical procedure. He has porphyrin staining or “red tears” around his eyes, nose, and medial forepaws. His incision appears swollen and painful. He has not been grooming. This animal should receive treatment to alleviate his pain and distress.
Strategies

The results of the systematic clinical exam described in this course should be documented in a study record for animal health.

When animals are found to be in pain or distress, appropriate individuals should be contacted (i.e., veterinary staff and investigators). Determining the appropriate response involves a team approach with both scientific and veterinary input.

A strategy to manage the adverse effects of the experimental procedures should be addressed in the protocol. Possible treatments may include the administration of analgesics, antibiotics, warmth, fluid therapy, nutritional supplements, etc.

Pharmacological Treatment

A number of analgesic options are available. Refer to your institution’s veterinary staff for treatment recommendations. Generally you should consider the use of local anesthetics, opioids, and non-steroidal anti-inflammatory drugs (NSAIDs). The opioids are controlled drugs and may be dispensed from an animal facility pharmacy.

Some commonly used analgesics are:

- **Opioid:**
  - Buprenorphine hydrochloride
- **NSAIDs:**
  - Meloxicam
  - Carprofen
  - Flunixin
- **Analgesic:**
  - Acetaminophen derivatives

Practical Recommendations
A practical approach to using analgesics in rodents is to prepare a batch of doses for a population of animals over the period of a study.

1. First calculate the total fluid volume required to dose all animals.
2. Then make a solution of the analgesic at a concentration that will deliver the desired dose per aliquot administered.

This approach can be used to medicate the animals with analgesic only or it can be used as a combination with hydration therapy. Remember to adjust the analgesic concentration according to whether the fluid aliquots will provide for hydration therapy or not.

**Oral Analgesics**

If injections are not necessary (i.e., for hydration therapy), you may consider offering an analgesic orally. A common approach is to add the analgesic (usually an opioid) to a gelatin treat, such as grape jelly, jello, or various commercial doughs or gels. (Rodents may prefer berry flavors and may avoid artificial citrus flavors.) Your veterinary staff will be familiar with these techniques.

When administering a medicated treat, it is important to be sure that the intended animal (and not cagemates) eats the whole dose.

**Important Considerations**

If there is concern whether an analgesic may interfere with the experiment, conduct a pilot study to determine whether the analgesic may affect the study or not. Of course, this pilot study must be approved by your IACUC first.

An important consideration in the use of analgesics is to reassess the animal for pain as the analgesic effect wanes. Perform a clinical exam for signs of pain to determine if another dose is needed. For information on a record-keeping system, refer to the next lesson, Documentation of Post-Procedure Care.

**Monitoring and Treatment**

Previous lessons have discussed practical methods for conducting a clinical exam on rodents to assess morbidity, pain, and distress. This lesson addresses the documentation of exam findings and treatments. A records management system aids in documenting the status of your animals over time. And in cases when multiple staff take turns monitoring the animals, a record system facilitates good communication among all persons involved in the care and use of these animals.

Photo:

The image below shows cages of rats that recently underwent surgery. The cages have been affixed with a “watch” card so that it is easy to find these cages on the rack when a person enters the room. Each cage card also corresponds to a 5”x8” medical record in a desk just outside the room. (There is a fresh orange for added nutrition; be careful not to leave fruit in a cage more than 12 hours as it will spoil.)
Components of a Record System

Typically, there are three components to a record system.

1. Cage identification system:
   Cages to be monitored should be flagged to help an observer quickly locate the cages to be checked among all others in the animal room. Once the animals are no longer being treated, a different cage flag can be used to indicate the need for a later recheck of these cages. Colored stickers, hanging tabs, or index cards may be used. Consider a color-coded system for distinguishing the type of monitoring.

2. Health record:
   A health record is used to document the clinical observations and physical exam findings. Records may be maintained for individual animals or a cage of animals. Consider using an index card, which can be kept in the cage card-holder throughout the monitoring period. A scoring system for clinical signs can be incorporated into the health record to provide both an efficient way to track the animals’ clinical profiles over time and to compile numerical data useful for scientific purposes. For more information, refer to the next page, Scoring Systems for Clinical Exam Data.

3. Record archive:
   It is helpful to archive animal health records so that they are accessible for routine use, for example in a procedure area adjacent to an animal room. The archive can be organized with a section of current cases. Staff who monitor the animals should routinely check this file before entering the room. This system allows for animals to be checked on a frequency that is appropriate to the condition – daily or weekly, for example.

Scoring Systems for Clinical Exam Data

A defined scoring system of clinical parameters is a valuable aid for monitoring animal morbidity. The clinical parameters and scoring standards should be appropriate for the animal species and the disease model. This system can facilitate the decision to intervene to allay an animal's pain/distress, e.g., to administer treatment or euthanasia.

If appropriate clinical parameters are not known for a particular disease model, you can perform a pilot study on a small number of animals to:

- Characterize the relevant clinical parameters;
- Define the time course of the disease and related critical events;
- Refine the endpoints; and
- Determine the timing and frequency for animal monitoring.

**Scoring Systems: Guidelines**

Some practical guidelines in developing a scoring system are to:

- Identify the clinical sign or signs that can be used to recognize the need for immediate euthanasia.
- Over successive studies, be mindful that the scoring system may need refinement. New and useful assessment methods may become available. Or, the clinical profile may change and require assessment by other parameters.
- Collect data on the numbers of animals that are euthanized vs. die unexpectedly. These data will help you refine the scoring system to minimize the number of animals dying from the experimental procedure without benefit of euthanasia.
- Publish your scoring system so that others may refine their methods based on your work.

A scoring system can be incorporated into the health record. This composite record can track the animals’ clinical profile and document the administration of treatments.

**Summary**

Good science requires good animal care. Animals that are in poor condition, discomfort, or pain are poor research subjects if such problems are extraneous to the objectives of the research. The impact on the animals’ physiology can alter the outcome of the research data. In these cases, animal well-being supports the integrity of the research.

In studies where animal morbidity is an expected outcome of the procedure (i.e., in a disease model when clinical symptoms are manifested), humane experimental endpoints should be established that do not conflict with the scientific objectives. The use of humane endpoints often benefits research by allowing the pre-mortem collection of biological samples. Using pre-established endpoints can avoid spontaneous death that results in loss of tissue due to post-mortem autolysis.

The strategies described here for assessing animal well-being and pain or distress are guidelines that can assist you in developing animal assessment methods that are appropriate for your experimental procedures.

Alleviation of pain and distress in animals is not achieved solely by the use of analgesics. Experimental procedures offer many opportunities for enhancing the animals’ well-being by the refinement of procedures to reduce the severity of injury or stress and by the provision of supportive care. Many such refinements were described in this course. Using a system to assess animal well-being will help document the improvements in technical procedures and the benefits from supportive care.
References


X. Rodent Surgery

This section is adapted from the handout written by Marcel I. Perret-Gentil, DVM, MS University Veterinarian & Director Laboratory Animal Resources Center, to accompany a hands-on rodent surgery workshop taught at The University of Texas at San Antonio

A. Application of Aseptic Technique and Perioperative Care

Regulations and Guidelines

- The Guide for the Care and Use of Laboratory Animals (National Research Council) states, “The relative susceptibility of rodents to surgical infection has been debated; available data suggest that subclinical infections can cause adverse physiologic and behavioral responses (Bradfield et al 1992; Cunliffe-Beamer 1990) that can affect both surgical success and research results.”
- According to OLAW guidelines for rodents a dedicated facility is not required, but surgery must be performed using aseptic technique.

Justification for Applying Aseptic Technique in Rodent Surgery

Importance of maintaining asepsis (NRC Guide for the Care and Use of Laboratory Animals):

- Although mice and rats have been touted as being resistant to post-surgical infections, the literature contains numerous articles that document how subclinical infections such as Pseudomonas aeruginosa, Corynebacterium kutscheri, mouse hepatitis virus, or Spironucleus muris can become clinical diseases following stress or immune suppression (Foster, et al., 1982).
- Historically, researchers have performed surgery in rodents in a non-aseptic manner. However, experimental evidence has been obtained to suggest that infections take a subclinical profile in rats and mice. Improvement in post-op recovery by increased food/water consumption due to implementing aseptic surgical technique has also been documented (Cunliffe-Beamer, T.L., 1972-73. Cunliffe-Beamer, T.L. Biomethodology, 1983). Experimentally induced wound infections in rats were not associated with gross clinical or obvious behavioral signs (Bradfield, Schachtman, McLaughlin, Steffen). Subclinical infections can lead to behavioral and physiological changes (Behavioral and Physiologic Effects of Inapparent Wound Infection in Rats, Lab. Animal Science, 42 (6), 572-578, 1992. Errata, Vol 43 (2), 20, 1993.)

It is unsafe to assume there is anything special, in either way, about the resistance of rodents to infections. Rodent models have been used for antibacterial research in which rodents have been used to model human bacterial diseases, including surgery related conditions. This fact would suggest that there might be no differences between rodents and other mammalian species, including humans, in the development of infections, including postsurgical infections (Morris T., Laboratory Animals, 1995, Vol 29, page 26).

Definitions

- **Sterile**: Free from all living microorganisms and their spores.
- **Asepsis**: Very insignificant numbers of microorganisms. A condition in which living pathogenic organisms are absent; a state of sterility.
- **Aseptic surgery**: The performance of an operation with sterile gloves, instruments, etc., and utilizing precautions against the introduction of infectious microorganisms from the outside environment.
- **Contaminated/Colonized**: Bacteria / microorganisms present (<10^6/gram tissue). Clinical signs may or may not be present. Immune response may be able to ward off infection.
- **Infected/Sepsis**: Bacteria/microorganisms present (>10^6 / gram tissue). Clinical infection is evident and cultures are positive.
- **Sterilization**: The process whereby all viable microorganisms are eliminated or destroyed. The criterion for adequate sterilization is the failure of organisms to grow if a growth-supporting medium is supplied.
- **Disinfection**: The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.
- **Major Surgery**: Any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation).
- **Minor Surgery**: Any surgical intervention that does not expose a body cavity and causes little or no physical impairment (such as wound suturing; peripheral-vessel cannulation; such routine farm-animal procedures as castration, dehorning, and repair of prolapses; and most procedures routinely done on an "outpatient" basis in veterinary clinical practice).

**Good Technique includes:**

- Asepsis
- Gentle tissue handling
- Minimal dissection of tissue
- Appropriate use of instruments
- Effective hemostasis
- Correct use of suture materials and patterns

**Skills are Practiced, Developed, and Refined**

Wrong Instrument Holding
Special Considerations

- Rats and mice have a high surface area to body volume ratio and rapid metabolism.
  - With high metabolic rate and limited fat storage, energy depletion can be stressful.
  - Pharmacological doses tend to be higher than in larger species.
  - Dehydrate faster per unit of time.
  - Lose body heat rapidly through hairless areas. Hypothermia during surgery is a frequent cause of intraoperative mortality.

- Surgical Stress:
  - The major responses to surgery are characterized by an elevation in plasma concentrations of catecholamines, corticosterone, growth hormone, vasopressin, renin, aldosterone and prolactin, and by a reduction in plasma concentrations of FSH, LH and testosterone. Plasma insulin and glucagon concentrations fluctuate. These hormonal responses to tissue trauma produce an increase in glycogenolysis and lipolysis, and result in hyperglycemia. The duration of the hyperglycemia varies, but after major surgery the response may persist for 4-6 hours. More prolonged changes in protein metabolism occur, leading to negative nitrogen balance lasting for several days. Even minor surgical procedures can produce prolonged effects.
  - Minimizing tissue trauma, preventing infection, controlling postsurgical pain and discomfort, and supporting the animal's nutritional needs will reduce the magnitude of the metabolic response to surgery. The purpose of a survival surgical procedure is to produce an animal model that is defined and that has the smallest degree of non-treatment variability. An important objective is to return the animal to physiological normality, or to a defined state of abnormality, as rapidly as possible.

Hemostasis

- It is important to minimize bleeding during surgery because:
  - Blood is a perfect growth media for bacteria
  - Blood loss leads to poor recovery and increases the chance of death
  - Blood loss increases post-op recovery time
  - Blood loss may introduce research variables

- To minimize blood loss:
  - Dissect along tissue planes
  - Do not cut across muscle
  - Identify, isolate, retract large vessels
  - Know the anatomy

Tissue Trauma & Contamination

- Trauma and infection negatively impact the animal and also serve as a confounding variable for experimental data. Diminish tissue trauma and infection by adhering to the following four principles:
  - “Surgery is gentle”: Rough tissue handling results in increased pain.
  - “Time is trauma”: Organ exposure to room environment is toxic to tissues. The longer the exposure the greater the trauma. Find the right balance between speed and quality of work. Incidence of infection increases 3 fold when surgery is longer than 90 minutes.
- “Wet tissues are happy tissues”: Avoid desiccation by maintaining tissues wet at all times with warm saline or lactated ringer’s solution (LRS).
- “The solution for pollution is dilution”: Infection occurs when the number (generally ∼10^6 infectious particles/gram of tissue in immunocompetent animals) of infectious particles overwhelm the animal’s immune system. Adhere as close as possible to the aseptic principles outlined in these notes to diminish the number of microorganisms in the wound site. If contamination occurs, dilute the contaminant with use of copious amounts of warm rinse solution (sterile saline or lactated Ringer’s solution).

Dealing with the Risk
- There is no such thing as a 100% guarantee or a risk-free environment
- Have to decide on the level of risk that is acceptable
  - Dependent on the type of procedure
  - Dependent on the complexity of the procedure
  - Dependent on the species
  - Dependent on the consequences of failure

Effect of Biomaterials
- Significantly decrease the number of bacteria needed to become clinically infected.
  - infection/sepsis
    - ∼1000 - 10,000 bacteria/microorganisms present (Paston et al. J Clin Micro, 1993)
    - clinically infected
    - culture positive

Preoperative Preparation
- Assess health status. Recommendations:
  - Five to seven day acclimation to animal facility, to overcome the stress of transportation.
  - Should be free of clinical signs of disease:
    - Appearance should include normal posture and movement, glossy coat, bright eyes.
Assess the character of respiration (no sneezing, coughing, or unusual respiratory sounds) and the cardiovascular status (bright pink coloration of ears and mucous membranes, albino animals).

- Normal intake of food and water
- Fasting rats and mice is generally unnecessary because rats and mice do not vomit, they do not have the risk of intra/post-op vomiting as in other species. If you will perform a surgery on the gastrointestinal tract, then you can fast the animals but briefly (a few hours). However, the reason for doing it should be considered carefully and weighed against the disturbance of normal metabolic processes needed for homeostasis. For example, starvation will not empty the stomach unless it is for more than 24 hours, but it will seriously deplete glycogen reserves in the liver (Behavioural and cardiac responses to a sudden change in environmental stimuli: effect of forced shift in food intake, Steenbergen JM; Koolhaas JM; Strubbe JH; Bohus B. Physiology and Behaviour 45, 729-733. Also Vermeulen JK, Vries de A, Schlingmann F & Remie R, (1997). Food deprivation: common sense or nonsense? Animal Technology, Vol 48, No 2, pg 45-54).

- Animal positioning
  - If limbs must be positioned for control of the surgical field, avoid placing excessive tension on the limbs, which may cause neural damage and shut off circulation.
  - Only tie down the limb(s) that need to be positioned. Remember the animal indicates that it may be becoming light by limb movement.
  - Avoid stretching the limbs into an unnatural position, which may traumatize joints as well as impair breathing.
  - If limbs must be tied down, apply strips of white tape around the carpal area and forelimbs. You can also tie a length of tape over the back, from carpus to carpus, to stabilize the forelimbs and torso.

- Never use the anesthetized animal’s body as a table. Do not rest your hands or your instruments on the chest or abdomen. External pressure interferes with respiration and blood circulation.

**B. General Preparations for Surgery**

The NRC Guide for the Care and Use of Laboratory Animals states: “Some characteristics of common laboratory-rodent surgery such as smaller incision sites, fewer personnel in the surgical team, manipulation of multiple animals at one sitting, and briefer procedures as opposed to surgery in larger species, can make modifications in standard aseptic techniques necessary or desirable (Brown 1994; Cunliffe-Beamer 1993). Useful suggestions for dealing with some of the unique challenges of rodent surgery have been published (Cunliffe-Beamer 1983, 1993).”

- Location
  - The elaborate operating suites mandated by the NRC Guide for larger species are not required for rats and mice.
  - What is necessary and required for survival surgery in these species is:
    - A clean, neat (uncluttered), disinfected area dedicated to rodent surgery for the duration of the procedure.
    - Free of debris and equipment not related to surgery.
A separation of functions of animal prep, operating field and animal recovery. These may be adjoining areas on a long bench top or better yet, animal prep is best when performed in a room separate from the room where surgery is to be performed. The rationale is to avoid contaminating the operating field with loose animal fur, splashes from incision site scrubbing, and bedding dust and fur from nearby cages.

- Avoid locations that are beneath supply ducts to minimize contamination from dust.
- Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence.

Surgical instruments should be autoclaved. Instruments must be double wrapped in linen or special paper or placed in a special metal box equipped with a filter before sterilization. Expiration dates should be printed on all equipment packs. If performing batch surgeries, i.e. using the same instruments on a series of animals, wipe them clean and resterilize the instrument tips (e.g. in a hot bead sterilizers between animals. You may need two sets of instruments to alternate use between animals.

- This method sterilizes only the tips of the instruments.
- Beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time (generally tips of instruments are exposed for 15 sec).
- Gross debris must be removed from the instrument prior to sterilization.
- Instrument must be allowed to cool before touching tissues.
- Best used for sterilizing instruments between surgeries.
- If you are doing a full day of batch surgeries, then use a fresh set of autoclaved instruments for the morning and the afternoon series.
- No more than five rodent surgeries is recommended by using this sterilization method. A new set of autoclaved instruments must be used for the next group of animals.
- Liquid sterilants (e.g. glutaraldehyde [Cidex])
  - If using cold sterilant solutions make sure instruments are exposed for the proper length of time specified by the manufacturer and expiration dates of solutions are observed.
  - Instruments must be removed from solution and rinsed with sterile water, saline, or alcohol.
  - Rinsed instruments must be placed on a sterile field.
- Organize the instruments in your surgical pack
  - Point all tips in one direction.
  - It is helpful to place them in the order used.
  - Between surgeries cover the tips of the instruments with sterile gauze.
  - Note that the space between the pack and the draped animal is not sterile; do not lay instruments in this space.

C. Animal Prep

- Animals waiting for surgery should be kept at a visual and olfactory distance from those animals undergoing surgery.
- Anesthesia
  - Isoflurane gas anesthesia administration through a precision vaporizer is generally considered the preferred method of anesthesia in rodents; however injectable anesthetics may also be used.
  - Gas anesthesia may be induced in an induction chamber or it may be preceded by an injectable anesthetic cocktail.
  - For maintenance of anesthesia, a gas mask or endotracheal tube may be used to deliver the anesthetic. Endotracheal intubation can be performed using a 14-18 gauge intravenous catheter in a rat and a 20-21 gauge IV catheter in a mouse, trimmed to the length between the nose and thoracic inlet. Specialized apparatuses and kits can be purchased to facilitate intubation of rats and mice.
  - Waste gas from anesthetic gasses must be actively scavenged and vented to an outside source.
- Protect the eyes: Anesthetized animals should have corneas protected with an ophthalmic ointment. Avoid touching the eye with the tip of the ointment dispenser as it may scratch the cornea.

- Hair Removal
  - Remove fur along the incision site with small clippers. Clip a generous area to ensure fur does not contaminate the wound and a sufficient area that can be disinfected around the incision site, but avoid taking off too much fur, because this will reduce the animal's ability to regulate its body temperature.
• Antiseptic preparation of surgical site:
  o The use of alcohol alone is not adequate.
  o Standard surgical prep consists of three alternating scrubs of a chlorhexidine and 70% alcohol. Although pictures in this presentation illustrate the use of iodophors (povidone iodine), its use is no longer recommended as it has been shown to be inferior to chlorhexidine.
  o Using a gauze sponge or cotton tipped applicator, cleansing should be done in a circular motion.
  o Begin at the center of the shaved area and work toward the periphery.
  o Never go back to the center with the same sponge.
  o Scrubs should be alternated between a chlorhexidine scrub and alcohol, ending with a chlorhexidine solution, NOT scrub. Scrub soaps are irritating to subcutaneous tissue.
  o Be careful not to excessively wet the animal as this can exacerbate hypothermia.

• Draping is necessary when viscera or sterile instruments may come in contact with unprepped skin and fur. Types of drapes that may be used are:
  o Surgical paper drape. It is inexpensive, autoclavable.
  o It may be precut or one in which you must cut a hole.
  o A disadvantage to paper drapes is that they usually cover the animal making monitoring difficult.
  o Plastic drapes offer the advantage of more visibility.
  o Transparent, self-adhesive drapes, provided that the animal’s body is dry (use sterile gauze to daub dry prepped skin).
  o Glad’s Press’n Seal provides a sterile, inexpensive, and effective method to cover the surgical field. Although this is a food/grocery item, it has been tested and results were negative for the presence of any microorganisms and organic material. The sticky part is placed on the (entire) animal, which allows easy monitoring due to the see-through nature of this material. Make sure the nose is exposed to avoid suffocation.
  o Sterile gauze sponges can also be used for draping.
  o Note: During surgery - Be careful not to get paper or cloth drapes wet. Wet material acts as a wick to pull bacteria through from the non-sterile surface below. When this happens instruments should be considered
contaminated.

- **Surgeon**
  - Wash hands with an antiseptic soap.
  - Use sterile surgical gloves, mask, clean lab coat.
  - When using powdered gloves remove powder with sterile saline. Powder from gloves is a foreign material that leads to foreign body reaction.
  - If performing batch surgeries replace gloves or rinse your gloved hand or fingers with chemical sterilant followed by saline rinse. Sterilant residues on gloves will be irritating to tissues and will increase the risk for local infection. Dry with a sterile towel. Put on new sterile gloves when these become uncomfortable, torn or puncture.

- **Donning surgical gloves:**
  - Open the package of gloves observing sterile technique.
  - Remember, the inside of the package is STERILE – exam gloves are not the same as sterile gloves.

    ![Donning surgical gloves images]

- **Donning surgical gloves procedure:**
  1. Don the gloves in such a way that prevents contamination of the outer surface of the gloves.
  2. One glove is lifted from the opened glove package by its turned down cuff.
  3. & 4. The glove is pulled on the hand with a rotating motion.

    ![Gloving instructions images]

  5. Place the gloved fingers beneath the cuff of the other glove.
  6. With the gloved fingers under the cuff, the glove is placed on the ungloved hand. The folded cuff protects the gloved hand from contamination.
  7. It is pulled over the cuff of the lab coat following insertion of the hand.
  8. The fingers are then slipped under the cuff of the first glove to pull it over the lab coat cuff.
• Maintaining Asepsis (ungowned)
  o Gloved hands should be held elevated above the waist and should 
touch only the surgical incision and sterile objects, i.e. sterile 
instrument tray, sterile drape.
  o Once gloved, do not touch or lean over a non-sterile area. Do not drop 
your hands to your sides. Do not touch gloves to your skin or clothes.
  o Always lift an instrument from a sterile pouch or sterile surface. Do 
not drag instruments over the pack/drape edges because they can 
become contaminated.
  o Do not allow surgical instruments to fall below the edge of the table. If 
an instrument does fall, the instrument is no longer considered sterile 
and should not be picked up and reused until resterilized.
  o Sterile surfaces are to be kept dry. Moisture can lead to contamination 
of the surgical area.

• Preventing Hypothermia and supporting normal body temperature during 
anesthesia. A major cause of surgical mortality is not always the surgery or 
the anesthesia but hypothermia. Body temperature drops precipitously 
under sedation or anesthesia. Low body temperatures can cause irreversible 
shock and death.
  o Rats and mice have a high surface area and lose body heat rapidly by 
conduction.
  o Animals should be provided with a heat source during the pre-, intra-
and post-operative periods.
  o Improper heating devices can also be very dangerous. Electric 
heating pads are not recommended for use with rodents as they 
have varying temperatures across the surface.
  o The safest device is a circulating hot water blanket.
  o Place the animal on insulating materials (e.g. bubble wrap or folded 
drape).
  o You can test the environmental temperature by placing a simple 
thermometer in the vicinity 
  o of the animal for the approximate duration of a surgery (only 1-2° 
higher than body temp is necessary).
  o It’s easier to maintain normal body temperature than to reheat a 
chilled animal. If the animal is allowed to chill, there will be a 
reduction in circulation and organ function.
  o If heat lamps are to be used, make certain that they are placed at a 
safe enough distance so as not to cause heat burns to the animals, 
drape material, etc.
  o Supplemental heat is especially important when using chemical fume 
hoods or biosafety cabinets because of the high air flow generated in 
these hoods.
D. Intraoperative Anesthesia and Analgesia:

Anesthesia is a state where all perceived sensations are absent. Because drug effect can vary, you must assess the depth of anesthesia prior to beginning a painful procedure such as surgery.

The depth of anesthesia and the level of analgesia must be adequate to prevent the animal from feeling any pain in response to a surgical stimulus. Before making an incision, squeeze both rear paws firmly (toe pinch reflex) 3-4 times, but without injuring it, to test the animal’s perception of sensation and pain. If the animal withdraws its leg or if respiration rate increases, then the anesthesia is too light. The front toe pinch reflex may not be reliable as the pain perception may be present in the absence of a front toe pinch reflex. Assess how much time elapsed from administering the anesthetic and compare that to the expected time of peak effect. You may have to wait longer for the anesthetic to take effect. Or, if surgery was delayed, the anesthetic may have worn off. If neither of these time factors may account for the inadequate anesthesia, it is possible that you may have to use a higher dose rate of your anesthetic.

Preemptive analgesia is the prevention of pain before it occurs. As an adjunct to general anesthesia, a local anesthetic is used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively and in healing. Preemptive analgesia is also accomplished by administering a systemic analgesic before the pain insult occurs (e.g. before the surgical incision is made). In general, analgesics are more effective when administered prior to surgery.

Much of the post surgical pain is the result of the sensations produced in the skin and body wall of the incision area. Anesthesia of the local nerves prior to incising these tissues will greatly reduce post-op pain and distress. When the skin and tissues are incised, local sensory nerves become excited and transmit impulses to the brain that are interpreted as pain. During general anesthesia, the animal is unconscious and is unable to perceive the neural stimulations from the incision site and so is unaware of painful sensations. However, when the anesthetic has worn off, the brain will process these neural excitatory impulses, which continue postoperatively for days until the incision is healed. The result is that the surgical wound is painful and sensitive to touch and movement.

If a systemic analgesic or a local anesthetic is infiltrated prior to the incision, it will block or diminish the sensory neuroexcitation caused by cutting the tissues. When the animal wakes up, it will have a reduction in sensory stimuli from the incision area, and pain of the surgical wound will be greatly decreased both initially and throughout the period of wound repair. Inject a local anesthetic subcutaneously to infiltrate it in the vicinity where the incision will be made. Allow a few moments for it to diffuse and take effect before beginning the surgery.
An effective and simple analgesic consideration (that does not require the use of DEA controlled drugs) would be to prepare a 50/50 mix of lidocaine 1-2% with 0.5% bupivacaine. This is inexpensive and easy to administer. The surgeon can infiltrate the incision area just prior to closure or better yet, prior to making the incision. Lidocaine provides almost immediate pain control for 20-40 minutes and bupivacaine provides longer pain control for up to 4-6 hours. Since bupivacaine tends to sting upon injection, we recommend injecting it after the animal is anesthetized. In general major surgery requires systemic analgesics as the lidocaine/bupivacaine infusion only provides pain management to the incision site. Lidocaine and bupivacaine doses should not exceed 10 and 6 mg/kg respectively. Higher doses may lead to heart arrhythmias.

E. DELIVERY OF INHALANT AGENTS TO RODENTS

The best method for the delivery of volatile agents to rodents involves the use of a precision vaporizer and an anesthesia chamber alone or in combination with a face mask appropriately sized for rodents. The rodent is placed within the chamber for induction at 4-5% isoflurane concentration. Once anesthetized, the animal is removed from the chamber with anesthesia maintained by delivery through a face mask at 1-3% concentration. Both chamber and mask delivery incorporate the use of a precision vaporizer for precise control of the concentration of anesthetic gas delivered to the patient. Because oxygen flow is required to volatilize the liquid anesthetic placed within the vaporizer, oxygen is also delivered to the patient and helps to maintain the blood oxygen saturation. Because fairly high fresh gas flows are required for either chamber or mask delivery, adequate scavenging of waste anesthetic gases is necessary to avoid exposure to personnel. In general, isoflurane anesthesia is superior to injectable anesthesia. Animals are more quickly induced and recovered, and close to 100% of the gas is eliminated through the lungs without being metabolized, (<1% of isoflurane is metabolized). This allows for greater control of the anesthetic depth and tends to minimize experimental variables.

Precision vaporizers must be calibrated at the manufacturer’s recommended interval. In the absence of a manufacturer’s recommendation, calibration must be performed on an annual basis.

Calculating Vaporizer Oxygen Volumes:
Oxygen flow is calculated at 200-300 ml/kg/min but should never be kept at less than 500 ml/min, so for rats and mice maintain oxygen flows at 500 ml/min (0.5 L/min).

F. ANESTHETIC MONITORING OF RODENTS

Parameters that can be used to assess the depth of anesthesia in rodents include:
- recumbency and loss of purposeful movements
- muscle relaxation
- lack of vocalization
• loss of response to aversive stimulation (e.g. pinching the toes)

In most instances, cardiovascular and respiratory assessments are limited to observations of chest wall movement to determine respiratory rate and palpation of the apical pulse through the chest wall.

Because the ratio of body surface area to body mass is greater in rodents than in larger species, thermal support is critical to the successful recovery of rodents from anesthesia. Body heat may be dissipated from the tail, soles of the feet and ears with a resultant profound decline in the core and surface body temperature. This hypothermia may, in turn, lead to a decline in both anesthetic metabolism and any urinary excretion of the anesthetic agent.

**G. SUPPORTIVE CARE OF ANESTHETIZED RODENTS**

Methods to minimize heat loss to the environment during anesthesia of rodents include increasing the ambient temperature of the operating room; placement of a thermal blanket (e.g. recirculating warm water blanket) or drape between the animal and the stainless steel operating table; use of heat lamps (carefully placed!); minimization of organ exposure from body cavities during surgery; recovery of the animal on a warming blanket or within a temperature-supported cage; administration of warmed subcutaneous or intraperitoneal fluids before, during or after the anesthetic episode; housing on bedding during recovery to provide thermal insulation; and recovery with cage mates to permit animals to huddle together and thus provide thermoregulation. Do not place an unconscious rodent in a cage with an awake one as the alert animal will tend to mutilate the anesthetized rodent.

Rodents have high energy requirements due to their small size and high metabolic rate, yet they have minimal fat reservoirs which can be mobilized to supply needed energy (more so in mice). Nutritional support is critical upon recovery to avoid hypoglycemia. Nutritional support can be provided by simply providing a high-quality pelleted rodent diet as soon as the animal has recovered sufficiently to ambulate and eat (remember - rodents do not vomit so pre-anesthetic fasting is not typically performed).

Fluid deficits can be corrected by subcutaneous or intraperitoneal injection of warmed saline, Lactated Ringers solution or replacement fluids.

Because rodents are frequently anesthetized with injectable agents that inhibit blinking (e.g., ketamine), ocular lubrication is important to protect against corneal ulceration.

**H. INTRAOPERATIVE CARE**

- Monitoring
  - Anesthetized animals must be monitored during the procedure to assure they stay in the proper anesthetic plane.
  - The anesthetic plane can be assessed by pinching the toes, tail or ears of the animal for reflex response.
- Any reaction of the animal indicates the animal is too light and should be given more anesthetic.
- The color of the mucous membranes and exposed tissues, such as the pink soles of the feet, are easy to monitor. Bright pink and red tissue indicates adequate tissue perfusion. Pale, grey, or blue, indicates the animal is not receiving enough oxygen and restricted blood flow is keeping the tissue from being properly perfused.
- Respiratory pattern and frequency will also give an indication of anesthetic depth.
- Core body temperature can also be monitored in rats and mice.
- Pulse oximetry can be used in to monitor pulse and oxygenation.
- Electrocardiograms can also be used. **Respiration** – animal turns “blue” (hairless areas) if hypoxic.
- Evaluate the need for delivering oxygen...no special equipment is required. A tube delivering oxygen from a tank (turned to low flow) can be taped onto the table in the vicinity of the animal’s nose. Alternatively, a face mask may be made from a syringe case or syringe barrel.
- Maintain airway patency. Be careful in positioning the animal’s head and neck. Prevent blockage of the respiratory passages by blood, mucus, other material.
- If respiration rate falls progressively: If surgery is in progress, assist ventilation by gentle compression of the chest. If surgery is complete, administer an anesthetic antagonist (if appropriate) or a respiratory stimulant (e.g. doxapram). **Cardiovascular function** – the animal’s hairless areas (normally pink) turn “white” if tissue perfusion is poor.
- Assess the cause of cardiac impairment.
  - **Anesthetic overdose** – if appropriate, use an antagonist or an anticholinergics (e.g. atropine).
  - **Hypothermia** – greatest cause of rodent surgical mortality.
  - **Hemorrhage** of 3-4 ml in a 200 g rat will cause irreversible shock.
    - Surgical technique to minimize blood loss.
    - Blood transfusion – Ideal for inbred strains; no cross-matching necessary (keep a donor handy if the risk of hemorrhage is high).
      - **Outbred strains** - no problem likely when transfused once.
    - Blood volume is approximately 70 ml/kg. Hemorrhage and loss of 10% volume is tolerable, but 20-25% loss will cause shock.
- Consider using fluid therapy – to support cardiovascular function or to prevent dehydration.

Animals will have reduced food and water intake for 1-2 days after surgery. Providing sterile, warmed, physiological fluids (SQ, IP or IV) can be used to compensate for hemorrhage and reduction in water intake postoperatively.

- Recommended fluid replacement for mice is 17–33 ml/kg (~0.3–0.7 ml for a 20 g mouse) SC and 33 ml/kg (~0.7 ml for a 20 g mouse) IP; and for rats is 25 ml/kg (~5 ml for a 200 g rat) SC or IP. Sterile LRS or physiological saline warmed to body temp may be injected before the procedure if a prolonged recovery is expected or extensive hemorrhage may be likely.

- Or, infuse IV at a rate of 2 ml/100g/hr. A tail vein catheter may be placed before the procedure to be available for IV infusions if necessary.

- Consider whether the animal will have a reduced water intake for 12-24 hours post-op. Provide replacement fluids following guidelines in the previous section.

ECG – capability required for low amplitude and high rate monitoring. Tail cuff for blood pressure.

I. Surgical Technique:

- Minimize contamination of the operative field during surgery by restricting the movement of gloved hands and sterile instruments.
- Plan the incisions to avoid large vessels in the skin or body wall.
- Handle tissues gently and avoid excessive force in tissue retraction, which can cause necrosis.
- Avoid or minimize hemorrhage, but if it occurs, wick away blood with a sterile gauze sponge, Q tips or gel foam spears. Avoid using a wiping action, which traumatizes tissues and may cause renewed bleeding. Use a wicking or blotting action instead.
- If a wound becomes contaminated, use warm, sterile LRS or saline to irrigate and cleanse the area.

INSTRUMENT HANDLING: Generally scissors and hemostats are held with the thumb and the ring finger. Thumb forceps are held using a pencil grip technique.
The following pictures illustrate proper (and improper) instrument holding:

Safe surgical blade loading

Safe surgical blade unloading

Wrong instrument holding

Pencil grip technique for holding thumb forceps

Holding hemostats or scissors

Thumb & Ring finger

Holding the needle driver

Thumb & Ring finger

Palming the needle driver
SKIN INCISION: Placing tension on the sides of the incision with the non-dominant index finger and thumb while holding the scalpel handle with the dominant hand.

NEEDLE TYPE: If suturing with a needle, use the right type of needle for the type of tissue.

SOFT TISSUES – Use a tapered (round-bodied) needle on internal tissues (e.g. intestine, muscle, peritoneum). This type of needle passes atraumatically through soft tissues and allows them to “seal” behind the needle. Generally it is best not to use a cutting edge needle in soft tissues because this type of needle would tear the tissue, undermining the suture line, and it is more likely to cut through blood vessels leading to more hemorrhage in vascular tissues (e.g. muscle).

SKIN – Use a cutting edge needle on the skin (cutting or reverse cutting needle). The dermis has tough fibrous tissue. To pass a needle through it, cutting edges are needed to slide the needle through the skin. This minimizes trauma and irritation to the skin. As a result, the animal will be less likely to self-traumatize the sutured incision. On the other hand, if a tapered needle were used, the needle would have to be tugged through. The tugging and stretching of the skin would increase soreness of the skin wound.

Swaged-eye needles impose less trauma on tissues than do threaded needles.
ARMING THE NEEDLE:

Load the needle in its middle third (the loading zone)

Hold the needle with the tip of the needle holder with the needle perpendicular to the jaws of the needle holder

Holding the needle too close to the site of suture attachment will result in needle bending.

SUTURE MATERIAL:

- Use the right kind of suture material for the type of tissue.
  - Internal layers – Use an absorbable material, unless permanent ligatures are needed. Example material: Vicryl, PDS, Dexon, Maxon, sizes 3-0 and 4-0 in a rat; 4-0 and 5-0 in a mouse. Silk is frequently used for cardiovascular procedures.
  - Skin layer – Use a nonabsorbable monofilament sutures in skin (Prolene, nylon, stainless steel), wound clips, staples and/or tissue glue.
    - DO NOT use braided sutures, like “silk” because they tend to wick bacteria and tissue reaction and infection. This raises the chances of animal self-trauma.
    - Sizes 3-0 and 4-0 in a rat; 4-0 and 5-0 in a mouse.
    - Stainless steel (SS) to suture skin may be assembled inexpensively by purchasing a spool of 30 ga SS orthopedic wire.
      - A designated length of SS wire is threaded through a 22 ga needle.
      - The needle is bent at ~120° to secure (kink) the wire in the needle.
      - The hub of the needle broken away and discarded.
      - The suture and needle must be autoclaved before using in survival procedures.
      - When closing skin, two throws are enough to secure the suture.

SUTURE LAYERS AND PATTERNS:

1. Body wall (abdominal) – The suture line should be a simple, interrupted pattern, using absorbable suture material. A continuous pattern may also be used but it has some drawbacks. The body wall layer is an important one because it must take the tension in the body wall caused by animal movement, and so this layer must hold fast against tension. If a continuous suture line were used, and if a knot slipped or the suture broke, then the entire incision would dehisc.
2. Subcutaneous tissue – The suture line should be in a single continuous pattern, using absorbable suture. This should be used in larger rats which have a sizeable amount of subcutaneous tissue. It is generally not used in mice. Closing this layer collapses the potential space between tissue layers, preventing a seroma and abscess from forming. The subcutaneous layer will not have the tension of the body wall, and so that suture strength is not needed. Therefore, the continuous pattern can be safely used for its advantage of speed in suturing.

3. Skin – The suture line should be in a simple, interrupted pattern, for the same reasons as for the body wall layer. Use a nonabsorbable/monofilament material.

- Insert the needle about 5 mm from the wound margin.
- Space interrupted sutures (or clips) about 5-8 mm apart.
- Don’t cut suture so short as it can unravel later.

- A lot of rodents gnaw at externalized sutures so a buried suture line or wound clips are recommended.
- Cyanoacrylate skin glue (e.g. Vetbond, Nexaband, Dermabond) can be used for non-tension bearing wounds to appose skin edges for small incisions or to reinforce skin edges between sutures. Don’t bathe the skin wound because animals are likely to self-traumatize the area if there’s glue residue on the skin surface. Carefully place a tiny drop via an applicator tube onto the subcutaneous tissues or right over the skin. Use a probe to push the opposing edges of skin together, margin to margin. Avoid getting adhesive on the fur, or else the animal may later open up the wound in the process of removing the glue from its fur.

4. Closure – Proper apposition:

- Restore alignment of the tissues.
- Balance adequate closure with too much suture. Suture is a foreign body and too much can effect healing.
- Skin closure should be done by apposition of cut ends and not by overlapping of layers.
KNOT TYING/SECURITY

- Tie all sutures (any layer) with square knots with a third throw (3-4 throws). Square knots provide greater knot security against slippage when compared to slip knots. Prolene and nylon are slippery and may need 5 throws.
- Don’t cut knot strands too short. If cut too short, they will come undone later.
- If skin sutures are cut too long, the animal may chew on them and in so doing, remove the suture.

GRASPING TISSUES WITH THE THUMB FORCEPS

- Generally skin and body wall (linea alba) are grasped with fine rat tooth or Brown Adson forceps.
- Rat tooth forceps could be injurious to soft tissue.

HOLDING THE SUTURE TOO FAR OR TOO CLOSE

- When suturing hold the long end of the suture with your non-dominant hand at a comfortable distance from the knot (not too far, not too close).
IMPROVING SPEED AND ACCURACY WHILE DECREASING TISSUE TRAUMA

Stabilize your hands on a towel, animal or table to minimize trembling

Note the comfortable, well-supported & rested position of the hands, while using the middle, ring & pinky fingers to stabilize the instruments & hands

Hold the long suture strand with the non-dominant thumb and index finger creating a “V” between them. Hold the jaws of the needle driver on the “V” to improve your moves

Point the tip of the thumb and index towards the suture knot and incision while the “apex” of the “V” points away from the incision

Synchronize the dominant and non-dominant hands to move the jaws of the needle driver towards the short tail of the suture

Grasp the short tail of the suture with the jaws of the needle driver

Use the dorsal surface of the non-dominant middle finger (distal phalanx) to pull on the long suture strand while pulling on the short strand with the needle driver

As above, grasp the short tail of the suture with the needle driver using the “V” created by the non-dominant thumb and index to stabilize the jaws of the needle driver

Use the palmar surface of the non-dominant middle finger (distal phalanx) to pull on the long suture strand while pulling on the short strand with the needle driver

Minimize excessive tissue trauma with the suture and forceps. Avoid excessive traumatic pull on tissue while holding the suture...Make your moves fluid and smooth.
DEHISCENCE – Suture lines coming undone.

- The animal will chew and remove sutures if they are irritating.
- Be aware whether the suture strand will poke a body part or fold of skin. In skin fold areas, a suture strand may jab the skin and cause irritation. This may occur with monofilament nylon, because the cut end is hard. Skin fold irritation may be avoided by altering the placement of the sutures, changing the length of suture strands or by softening the suture material with daily applications of petroleum jelly to the suture end only.
- Avoid drawing sutures too tight. Wound margins normally become moderately edematous. Tight sutures will strangulate tissue and be painful. Overtightening skin sutures is the most common reason for animals removing their stitches.
- Maintain good aseptic technique. Infection macerates the wound margin and causes sutures to loosen and fall out.

SUTURE REMOVAL

Whether using sutures or staples, these must generally be removed from the skin at 10-14 days after the surgery. The time will vary depending on the surgical site. If sutures or clips are not removed, they will become embedded in the skin and will cause irritation and possibly infection. At some point, the animal will chew and remove the sutures or clips because of the irritation. Remove sutures by lifting the knot and cutting the suture portion that was under the skin prior to pulling, then cut as close as possible to the skin as possible to avoid contaminated suture from entering the skin.

Prophylactic Antibiotics

- Rarely needed if true aseptic principles are adhered to, but if used, keep the following guiding principles in mind:
  - Antibiotics may make a 3rd rate surgeon into a 2nd rate surgeon, but they will never make a 1st rate surgeon out of a 2nd rate surgeon
  - Administer before surgery so high tissue levels are present at the time of surgery
  - Pre-op antibiotics can reduce the risk of infection in complicated surgeries
  - Generally there is no need for antibiotics after surgery
  - Use broad spectrum antibiotics
  - “Good antibiotics do not make up for poor surgery.” The “Guide” states that the application of prophylactic antibiotics is not a substitute for the practice of proper aseptic surgery.
- In guinea pigs, rabbits and hamsters an inappropriate antibiotic can cause fatalities.
• If possible, use anesthetic/sedative antagonists to recover the animal more quickly from anesthesia.
  o Yohimbine or atipamezole – to reverse xylazine and medetomidine respectively.
  o For respiratory depression – doxapram, 5-10 mg/kg IV or IP. Retreat as necessary (15 min intervals).

• Continue providing a source of heat until the animal is conscious enough. Animals are considered conscious enough when the righting reflex has returned. The righting reflex is tested by placing the animal on its side or back. If the animal places itself on its four feet, then the righting reflex has returned.

• Provide clean bedding to avoid wound contamination.

• Assess food and water intake for several days.
  o Animals may not drink for one or more day post-op and will therefore dehydrate. Recommended fluid replacement for mice is 17–33 ml/kg SC and 33 ml/kg IP; and for rats is 25 ml/kg SC or IP.
  o If animals are dehydrated, provide further fluid therapy and consider doing so by IV infusion. However, since IV administration is difficult to do in rodents, SC or IP administration provide a good alternative. Test for dehydration by pinching and pulling the skin just cranial to the shoulder blades into a tent and then releasing it (“tenting the skin”). If normally hydrated, the skin will snap back towards the body. If dehydrated, the skin will fall slowly into place.
  o Daily weighing is a sensitive method of monitoring the animal. While subtle changes in activity or appetite may not be observed, changes in weight will be quickly detected. Some analgesics depress appetite and must be differentiated from that which occurs if an animal is not feeling well.
  o Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat.

• Environment: Rats and mice prefer low lighting and quiet places to hide. Provide some cover: place a drape over the cage.

• Observe the animals for signs of pain or distress postoperatively.
  o Remember that rats and mice are nocturnal and are less active during the day, making it difficult to assess their behavior at times of less than peak activity.
  o Compare posture and activity with normal animals.
  o Rats and mice are able to mask pain. Therefore, pain may be evident in altered behavior or it may not. Most evident will be a reduction in food and water consumption.
  o If the procedure is likely to produce pain in humans, it should be assumed to be painful in animals and should be treated with analgesics.
  o Abnormal signs – any may be seen: hunched posture, ruffled fur, red staining of eyes and nares (rats), vocalization, greater or less tissue coloration, greater or less activity, resents being handled, reluctant to be moved, abnormal gait, aggressiveness, low water or food intake, subtle weight changes.
  o The first 24 hours are critical for pain management.

• Neonates:
  o Neonates or animals recovering from prolonged surgeries can suffer from hypoglycemia.
  o These animals can benefit from administration of oral glucose.
  o Glucose should never be given SQ or IP.
In general the following guidelines should be used during the immediate post-op period:

- Never leave unconscious animal unattended (monitor continuously until return of righting reflex, i.e. sternal).
- Analgesics must be given as stated in the protocol.
- Supportive therapy as needed (e.g. supplemental heat, warmed SC or IP fluids).
- Monitor incision daily ≥ 5 days.
- Notify your veterinary if any complications appear.
- Suggested 3x5 card to use for post-op monitoring.

---

**FRONT**

**RODENT POST-SURGICAL CARE CARD**

Surgery Date ____________________________

Suture/Clips Removal (date) ____________________________

Protocol # ____________________________

Animal ID ____________________________

PI ____________________________

Contact Ph # ____________________________

Surgery Site (e.g. abdomen, hind leg, head, etc.) ____________________________

Drugs, Doses and Routes (Anesthetics, Analgesics, Antibiotics, fluids, etc.) ____________________________

---

**BACK**

Post-operative monitoring/notes must be continued minimum of 5 days after surgery

Front/Time: ____________________________

Remarks (Infection, Pain, x's, Behavior, etc.) ____________________________

Initials ____________________________

CDLAR063
• Analgesia

- Types of analgesics: Opioid and nonsteroidal anti-inflammatory drugs (NSAID).
- Choice need not be limited to one or the other. Both can be given and are additive in effect.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Rat</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine*</td>
<td>0.05-0.1 mg/kg SC q 12 h</td>
<td>0.01-0.05 mg/kg SC, IV q 8-12 h</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.1-0.25 mg/kg PO</td>
<td>Opioid</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>1-2 mg/kg SC q 4 h</td>
<td>1-2 mg/kg SC q 2-4 h</td>
</tr>
<tr>
<td>Morphine</td>
<td>2-5 mg/kg SC q 4 h</td>
<td>2-5 mg/kg SC q 4 h</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5 mg/kg SC q 24 h</td>
<td>5 mg/kg SC q 24 h</td>
</tr>
<tr>
<td>Carprofen</td>
<td>5 mg/kg SC q 24 h</td>
<td>5 mg/kg SC q 24 h</td>
</tr>
<tr>
<td>Flunixin</td>
<td>2.5 mg/kg SC, IM q 12 h</td>
<td>1.1-2.5 mg/kg SC, IM q 12 h</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1-2 mg/kg SC, PO q 12 h</td>
<td>1-2 mg/kg SC, PO q 12 h</td>
</tr>
</tbody>
</table>

* Buprenorphine is the only opioid with long duration effect in rodents.


**NSAID dosing caution:**

1. Ensure that animals are adequately hydrated (skin pinch test, or serum Total Protein test) before administering an NSAID to avoid renal damage.

2. NSAIDs must be used with caution beyond 3 days as it may have deleterious effect on the gastrointestinal mucosa. This may be especially true when using ketoprofen and flunixin.

**Opioid dosing caution:**

1. Opioid agents enhance sedative and respiratory depressive effects of anesthetics.

2. For rodents anesthetized without respiratory support (intubation, ventilation and oxygen supplementation), you may wish consider opioid administration until the end of surgery.

3. In this case, an NSAID may be the preemptive analgesic of choice

4. If ventilatory support can be provided and an opioid is used as a preemptive analgesic agent, expect to reduce the dose of anesthetic agent (e.g. pentobarbital, isoflurane) by 30-50%.

**Oral dosing caution:**

1. Animals should be acclimated to oral medications before the surgery. When added to the drinking water, rodents will initially refuse to drink until they become adjusted to the flavor, which could be disastrous postoperatively.

2. When used in drinking water, analgesics should generally be administered 5-7 days prior to the anticipated pain insult.

3. Consideration to the use of analgesics in drinking water must take into account that postoperatively, animals may decrease fluid intake and may therefore not receive the intended analgesic dose.
Topical Anesthetic — Preemptive Analgesia:

Don’t forget preoperative (preferred) or intraoperative infiltration of the incision site and underlying tissues with 1-2% lidocaine/0.25-0.5% bupivacaine (50/50 mix by volume). This may prevent the need for any follow-up analgesic treatment or at least delay it for up to 6 hours. You may also choose to administer preemptive analgesics systemically but be careful with the potentiated side effects the analgesic may have with the anesthetic.

<table>
<thead>
<tr>
<th>Local Anesthetic</th>
<th>Onset</th>
<th>Duration</th>
<th>Do not exceed (toxic dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine (xylocaine)</td>
<td>1-3 min</td>
<td>20-40 min</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>~20 min</td>
<td>4-6 hours</td>
<td>6 mg/kg</td>
</tr>
</tbody>
</table>

Adjuvants: Adding epinephrine (1:50,000 to 1:200,000) to plain solutions of local anesthetics just before administration shortens the onset time and prolongs the duration of action. A 1:200,000 dilution is obtained by adding 0.1 ml of 1:1000 epinephrine (with a tuberculin syringe) to 20 ml of local anesthetic. Epinephrine **should not be used for peripheral nerve blocks in areas with poor collateral circulation e.g., digits, tails. Use caution if patient has cardiac problems.**

The Argument for NOT Using Analgesics — it may affect my research results…!!

**MYTH OR TRUTH???

In trying to justify precluding analgesics (because of their “real” or “perceived” potential effects they may have on the animal’s immune system), the researcher should consider the effects that unalleviated pain may have on the animal and the experimental results.

The investigator should consider that pain itself affects the animal’s overall physiology and immune system. Such effects are can be significantly more profound than the effect that analgesics themselves may have on the research results.

Some of the effects of unalleviated pain may include:

1. Activation the sympathetic nervous system
2. Increasing cardiac output
3. Increasing systemic vascular resistance
4. Increased blood pressure
5. Increased oxygen demand
6. Vasoconstriction of the coronary arteries
7. Ischemia of the spleen
8. Adverse renal effects
9. Increases the output of hormones like aldosterone
10. Increases sodium and water retention
11. **Release of endogenous glucocorticoids, which are immunosuppressive**
<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Indications &amp; Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>90-120 mg/kg</td>
<td>IP, IM</td>
<td>Surgical anesthesia</td>
</tr>
<tr>
<td>Xylazine</td>
<td>10.00 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>50-75 mg/kg</td>
<td>IP</td>
<td>Moderate surgical anesthesia. Not for major surgery</td>
</tr>
<tr>
<td>Medetomidine</td>
<td>1.00 mg/kg</td>
<td>IP</td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>30.00 mg/kg</td>
<td>IM</td>
<td>Surgical anesthesia</td>
</tr>
<tr>
<td>Xylazine</td>
<td>6.00 mg/kg</td>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>Acetylpromazine</td>
<td>1.00 mg/kg</td>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>Tribromoethanol</td>
<td>125-250 mg/kg</td>
<td>IP</td>
<td>15-45 min surgical anesthesia, 60-120 min sleep time</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>40-85 mg/kg</td>
<td>IP</td>
<td>Surgical anesthesia</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>2.1 mg/kg</td>
<td>IP</td>
<td>Xylazine antagonist for reversal of ketamine/xylazine anesthesia</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>1 mg/kg</td>
<td>IP, SC, IV</td>
<td>Medetomidine antagonist for reversal of ketamine/medetomidine anesthesia</td>
</tr>
<tr>
<td>Acetylpromazine</td>
<td>2-5 mg/kg</td>
<td>IM, IP</td>
<td>Sedation</td>
</tr>
<tr>
<td>Diazepam</td>
<td>5 mg/kg</td>
<td>IP</td>
<td>Sedation</td>
</tr>
<tr>
<td>Xylazine</td>
<td>10 mg/kg</td>
<td>IP</td>
<td>Sedation</td>
</tr>
</tbody>
</table>
Examples of Injectable Rat Anesthetics, Anesthetic Cocktails and Sedatives

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Indications &amp; Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>80-90</td>
<td>mg/kg</td>
<td>IP, IM Surgical anesthesia</td>
</tr>
<tr>
<td>Xylazine</td>
<td>8-10</td>
<td>mg/kg</td>
<td>IP, IM Moderate surgical anesthesia. Not for major surgery</td>
</tr>
<tr>
<td>Ketamine</td>
<td>75</td>
<td>mg/kg</td>
<td>IP Simple to moderate surgical procedures, e.g. laparotomy, certain stereotaxic procedures</td>
</tr>
<tr>
<td>Medetomidine</td>
<td>0.5</td>
<td>mg/kg</td>
<td>SC Extensive surgical procedures</td>
</tr>
<tr>
<td>Ketamine</td>
<td>31.25</td>
<td>mg/kg</td>
<td>SC Surgical anesthesia. For perfusion use 80-120 mg/kg</td>
</tr>
<tr>
<td>Xylazine</td>
<td>6.25</td>
<td>mg/kg</td>
<td>IP Xylazine antagonist for reversal of ketamine/xylazine anesthesia</td>
</tr>
<tr>
<td>Acetylpromazine</td>
<td>1.25</td>
<td>mg/kg</td>
<td>IP Medetomidine antagonist for reversal of ketamine/medetomidine anesthesia</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>40-50</td>
<td>mg/kg</td>
<td>IP Surgical anesthesia</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>2.10</td>
<td>mg/kg</td>
<td>IP Xylazine antagonist for reversal of ketamine/xylazine anesthesia</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>1.00</td>
<td>mg/kg</td>
<td>IP Medetomidine antagonist for reversal of ketamine/medetomidine anesthesia</td>
</tr>
<tr>
<td>Acetylpromazine</td>
<td>1-2</td>
<td>mg/kg</td>
<td>IM Sedation</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2-4</td>
<td>mg/kg</td>
<td>IM, IP Sedation</td>
</tr>
<tr>
<td>Xylazine</td>
<td>1-3</td>
<td>mg/kg</td>
<td>IM Sedation</td>
</tr>
</tbody>
</table>

References

12. Kohane DS; Sankar WN; Shubina M; Hu D; Rifai N; Berde CB. Sciatic nerve blockade in infant, adolescent, and adult rats: a comparison of bropivacaine with bupivacaine. Anesthesiology, 1998 Nov, 89(5):1199-208; discussion 10A.
15. Steenbergen JM; Koolhaas JM; Strubbe JH; Bohus B. Behavioural and cardiac responses to a sudden change in environmental stimuli: effect of forced shift in food intake. Physiology and Behaviour 45, 729-733.
XI. PRINCIPLES OF ANESTHESIA AND ANALGESIA

As stated in the Animal Welfare Act and the Guide, anesthetic, analgesic, and sedative agents should be used when necessary on animals utilized in biomedical research for humane and scientific reasons. These agents are used to control pain and distress. If the use of these agents would interfere with the objectives of the study then the procedures must be directly supervised by the responsible investigator in accordance with all regulations and guidelines governing these situations. Investigators requiring the non-relief of “pain or distress” in their protocol procedures are required to provide well documented evidence that relief of this pain will interfere with the research results. The use of these agents by investigators/technicians should be reviewed and approved after consulting with the institution’s veterinarian and Institutional Animal Care and Use Committee (IACUC).

Anesthesiology is not an exact science. Recommendations and dosages given in textbooks and formularies should be taken as guidelines. An investigator contemplating a procedure requiring anesthesia, tranquilization, or analgesia should not neglect the resource of the staff veterinarian who can often provide valuable assistance. In fact, the Animal Welfare Act requires that, "in any practice which could cause pain to animals...a doctor of veterinary medicine is consulted in the planning of such procedures."

There are many variables affecting an animal’s response to anesthesia. Because the absorption and biotransformation of drugs differs among species, it is nearly impossible to develop a single anesthetic or analgesic protocol that applies to all laboratory animals. Morphine can cause profound CNS depression in the rat and rabbit but can cause tremors and convulsions in mice and cats. The dosage of xylazine needed to sedate a ruminant is one-tenth that necessary to sedate a dog. These are but two of many examples. A common mistake is to extrapolate dosages across animal species or from man to animals. The strain of animal used is also a variable to consider. Some rat strains are sensitive to nitrous oxide. Some breeds of dogs (whippets and greyhounds) are more sensitive to barbiturates than other breeds. The size and even the sex of the animal can make a difference in the response to anesthetics. In rats, females are more sensitive to barbiturates but in mice, barbiturate narcosis lasts longer in males. The temperament of the animal can change the way it responds to a given agent. Some tranquilizers will cause a vicious dog to become even more difficult to handle.

Whenever possible, try a new anesthetic protocol in a limited number of animals before depending on it for surgical or painful procedures involved in an experiment. This allows determination of suitability for the anticipated protocol and allows necessary changes to be made before it effects the data being collected. It also facilitates familiarization with the anesthetic method to minimize problems later, when attention is often focused on surgical procedures or data collection.

Pay particular attention to the health of the animal before using it in an experiment. A pre-anesthetic checkup is a must. To minimize anesthetic risks, only use healthy animals and allow them to acclimate to the facility before an anesthetic procedure. Consider the general adaptation syndrome: alarm increases basal metabolic rate which may increase the amount...
of anesthetic needed; however, this is often followed by an exhaustion phase when less anesthetic is required.

Use the minimal degree of CNS depression necessary for the procedure that is compatible with the animal’s welfare. The degree of depression required for procedures such as radiographs or blood withdrawal is not the same as that needed for a thoracotomy or orthopedic procedure. Remember, during painful procedures, the use of paralytics without anesthesia is prohibited by law.

Consider if, and to what extent, the anesthetic protocol will affect the validity of experimental results and how it will react with other drugs being used. For example, if studying catecholamine effects, halothane should be avoided since its combination with catecholamines can cause severe cardiac dysrythmias.

Regard the conservation of heat as an integral part of anesthetic management. This is particularly important in small or young animals. A rectal thermometer can help monitor the animal’s body temperature. More sophisticated thermal monitors are also available. Maintenance of body temperature is enhanced through the use of external heat sources such as hot water bottles, thermal blankets, etc... Care should be taken to avoid thermal burns from external heating sources such as electric heating pads and heat lamps.

**General anesthesia is divided into stages and planes.**

These stages occur (when using inhalation anesthesia alone; other drugs added will modify these stages.

**Stage 1** (Induction, aka voluntary excitement). Excitement and struggling are common. Usually accompanied by ephinephrine release with associated rise in respiratory rate and heart rate.

**Stage 2** (delirium, involuntary excitement). Voluntary centers and loss of consciousness begin. Exaggerated reflexive responses to stimuli are common, as is vomiting (in species that can vomit). Breath holding may occur. Common hazard: self-injury.

**Stage 3** General Anesthesia

- Plane 1—Light anesthesia. Most reflexes (pedal, corneal, palpebral) are still present.
- Plane 2—Medium anesthesia. Most surgeries are conducted at this level. Muscles are relaxed. Most reflexes (pedal, palpebral, corneal) are absent.
- Plane 3—Deep anesthesia. Intercostal muscles are relaxed; ability to maintain respiration is endangered. Pupillary light reflex may be slow or absent.
- Plane 4—Too Deep. All muscles, including diaphragm & intercostal muscles, are paralyzed.

**Stage 4** Irreversible Anesthesia—respiratory arrest, followed by circulatory collapse. Death within 1-5 minutes.
When anesthetizing small rodents, particular care must be taken to avoid hypothermia. The airway is easily obstructed so be sure the neck is adequately extended and secretions are aspirated as necessary. A 6-hour fasting period may be necessary for some species. Water should not be restricted. Loss of the toe pinch reflex indicates anesthesia in the mouse. In the rat and guinea pig, the ear pinch is more sensitive. Rodents are difficult to intubate. If they are intubated, care must be taken to minimize dead space in the tubing.

Rabbits are probably the most difficult laboratory animal to anesthetize. Their respiratory center is particularly sensitive to anesthetics and a lot of individual variation in response exists. Rabbits should be fasted six hours prior to anesthesia. Water should not be restricted. The rabbit trachea is very delicate and rabbits are predisposed to pulmonary edema with prolonged inhalation administration. A normally small lung capacity combined with enzootic pulmonary disease further complicates the situation. The best indicator for surgical anesthesia is the loss of the ear pinch reflex. Intubation in rabbits is difficult due to lack of visualization of the larynx, but it can be mastered with practice.

INVESTIGATOR RESPONSIBILITY

1. Compliance with all regulations and guidelines.
2. The individual performing the procedure is responsible for the animal(s) care and use.
3. The following must be provided:
   • Consultation with the university veterinarian.
   • Review and approval of protocol for the procedure by the IACUC.
   • Training of all individuals using or caring for animals.
   • Proper use of analgesics, tranquilizers, and anesthetics.
   • Proper pre-operative, operative, and post-operative care.
   • Provisions for emergency care and euthanasia, if necessary.
4. Maintain adequate records.
SPECIES-SPECIFIC BEHAVIORAL SIGNS OF PAIN

<table>
<thead>
<tr>
<th>Species</th>
<th>Vocalizing</th>
<th>Posture</th>
<th>Locomotion</th>
<th>Temperament</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>Whimpers, howls, growls</td>
<td>Cowers, Crouches; Recumbent</td>
<td>Reluctant to move; awkward, shuffles</td>
<td>Varies from chronic to acute; can be subdued or vicious; quiet or restless</td>
</tr>
<tr>
<td>Cat</td>
<td>Generally silent; may growl or hiss</td>
<td>Stiff, hunched in sternal recumbency; limbs tucked under body</td>
<td>Reluctant to move limb, carry limb</td>
<td>Reclusive</td>
</tr>
<tr>
<td>Mice, rats, hamsters</td>
<td>Squeaks, squeals</td>
<td>Dormouse posture; rounded back; head tilted; back rigid</td>
<td>Ataxia; running in circles</td>
<td>Docile or aggressive depending on severity of pain, eats neonates</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Piercing squeal on acute pain</td>
<td>Hunched; faces back of cage</td>
<td>Inactive; drags hind legs</td>
<td>Apprehensive, dull, sometimes aggressive depending on severity of pain; eats neonates</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Urgent repetitive squeals</td>
<td>Hunched</td>
<td>Drags hind legs</td>
<td>Docile, quiet, terrified, agitated</td>
</tr>
<tr>
<td>Birds</td>
<td>Chirping</td>
<td>Huddled, hunched</td>
<td>From excessive movement to tonic immobility depending on severity of pain</td>
<td>Inactive; drooping, miserable appearance</td>
</tr>
</tbody>
</table>

ASSESSMENT OF PAIN IN THE LABORATORY SETTING

A fundamental approach to assessment of pain in laboratory animals does not begin with chemical or biological evaluations. The key to adequate assessment lies in the hands of the animal care personnel: technicians, laboratory specialists, and researchers. It is here that clinical observations and abnormal behavior should be recognized as possible identifying factors of pain in laboratory animals. It is therefore essential that all personnel involved in the care of animals be well versed in normal animal behavior patterns and that they recognize any deviation from the normal or usual pattern. The conscientious laboratory animal personnel performing daily routine functions should identify changes in personality, eating habits, physiological functions, etc. Such observations should be reported quickly to the clinical veterinarian or appropriate animal health care official. Good communication among all animal health care personnel is essential. Early recognition of abnormal signs or any deviation from usual daily animal performance can mean the difference between mild, moderate, or severe pain.
Anticipating when signs of pain may occur is an important part of minimizing and preventing unintended suffering in animals. This can be accomplished by a thorough knowledge of expected results of all experiments which are known or are likely to produce pain and suffering. In recent years, the field of laboratory animal medicine has established a protocol system that describes each animal experiment prior to its initiation. Clinical veterinarians should review each protocol for assessment of research which may cause pain, stress, distress, discomfort, or suffering to animals. This review also will reveal proposed drug usage which could interfere with or react with post-procedural pain medication. Review of protocols prior to performance and review of drug literature and analgesics known not to interfere with the experimental design or protocol can enhance treatment of post-procedural pain. Knowledge of the general responses of animals to a given procedure is important in the assessment and management of pain.

Knowledge of an animal’s disposition and normal physiological functions prior to execution of experimental protocols is extremely helpful in determining whether an animal is in pain. Aggressiveness, attempting to bite, hissing, and/or withdrawal can be interpreted as signs of pain. However, if such behavior was present prior to manipulation and is characteristic of the animal in question, then these indices are not necessarily indicative of pain or suffering. It cannot, however, be assumed that the animal is not in pain, and a thorough assessment for post-procedural pain should be performed. Comparison of pre- and post-procedural behavior may indicate that the animal is still growling, hissing, or attempting to bite, but movements or attempts to escape may be minimum to none. The importance of being aware of pre-procedural traits cannot be over-emphasized.

ASSESSING UNCONSCIOUSNESS AND DEATH

The level of consciousness and an animal’s ability to feel pain may be measured by testing the palpebral, corneal or "blinking" reflex. This reflex is absent in the cat and when curariform drugs or dissociative anesthetics are used in other species.

The signs of death in an animal are indicated by:
- a. cessation of respiration
- b. absence of a heartbeat or pulse - verify before disposal
- c. loss of reflex responses
- d. a flat or isoelectric electroencephalogram (EEG)
- e. total flaccidity of the muscles which is followed in minutes or hours by rigor mortis.
XII. EUTHANASIA

This section is adapted from the AVMA Guidelines on Euthanasia, 2013. A complete copy of this document can be found at https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

THE MEANING OF EUTHANASIA

The word "euthanasia" is derived from the Greek, eu-, well + thanatos-, death. An easy and painless death.

UNITED STATES DEPARTMENT OF AGRICULTURE

For the purpose of the Animal Welfare Act, "euthanasia" means the humane destruction of an animal accomplished by a method which produces instantaneous unconsciousness and immediate death without visible evidence of pain or distress, or a method that utilizes anesthesia produced by an agent which causes painless loss of consciousness, and death following such loss of consciousness.

GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS

For the purposes of the Guide for the Care and Use of Laboratory Animals, "euthanasia" is the procedure of killing animals rapidly and painlessly. It should be carried out by trained personnel using acceptable techniques in accordance with institutional policies and applicable laws. The method should not interfere with postmortem evaluation.

HUMANE CONSIDERATIONS

Primary Criterion for Evaluating a Technique
A method of euthanasia must cause loss of consciousness and have an initial depressive action on the central nervous system which will ensure insensitivity to pain without fear or anxiety. Death does not necessarily follow loss of consciousness of CNS depression.

Aesthetics Versus Humaneness
Some methods of euthanasia, such as decapitation or cervical dislocation, are not aesthetic to the observer but none the less are humane. A technique of euthanasia is considered humane if it causes CNS depression and insensitivity to pain. Pain recognition in an animal and man is dependent on impulses from pain receptors reaching the thalamus and cerebral cortex. If these structures are not functioning, pain will not be felt. Therefore, a technique of euthanasia is considered humane if this occurs. In the unconscious animal, stimuli that invoke pain will elicit reflex responses manifested by motor movement but this does not indicate cerebral pain reception.
Euthanizing an Animal at the End of an Experiment
Many animal experiments require data that is collected during a postmortem examination. Animals may be painlessly killed in order to harvest tissues or organs for in-vitro experiments. Endangered species or valuable animals are not generally killed but are recycled to other projects.

Euthanizing an Animal on Humane Grounds
Whenever an animal is in pain or distress which cannot be relieved, it must be painlessly killed even if the experiment is not complete. The decision of whether or not to kill an animal should rest with the professional judgment of a veterinarian. This authority has been granted to the attending veterinarian by the Institutional Official (IO) and the IACUC of this institution. If the investigator and veterinary staff cannot reach consensus on treatment, the veterinarian has the authority to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia.

Euthanizing an Animal for Disease Control
When an animal develops a disease and cannot be isolated or treated, then it should be painlessly killed for disease control.

SELECTION OF A TECHNIQUE OF EUTHANASIA
Criteria for Selecting a Technique
The method of choice of euthanasia will depend on:
- species of animal
- number of animals
- experimental purpose
- the operator and observer

A method of euthanasia should attempt to meet the following criteria:
- death without anxiety, panic, or pain
- restraint technique should not precipitate the above reactions
- minimizes the time to loss of consciousness and death
- will cause death when properly used
- safe for operator and observer
- minimizes undesirable physiological and psychological effects
- compatible with experimental requirements
- minimizes emotional effects on operator, observer
- "idiot proof," simple, and maintenance free
- minimizes sanitation problems and environmental contamination

Cervical Dislocation
Cervical dislocation is a technique of separating the skull and brain from the spinal cord by applying pressure posterior to the base of the skull and spinal cord. It is utilized to humanely kill mice and immature rats.
"The thumb and index finger are placed on either side of the neck at the base of the skull or a rod is pressed at the base of the skull. With the other hand, the base of the tail or hindlimbs are quickly pulled causing separation of the cervical vertebrae from the skull."

**Advantages**
- produces rapid unconsciousness and death
- tissues and blood not contaminated by chemicals

**Disadvantages**
- aesthetically displeasing
- limited to mice and immature rats

**Recommendation**
Acceptable only for poultry, other small birds, and mice and rats weighing less than 200 grams when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation. Institutional Animal Care and Use Committee must determine that personnel who perform cervical dislocation techniques have been properly trained and consistently apply it humanely and effectively.

**Decapitation with Guillotine**
Decapitation is the method of rapidly and completely severing the head from the body using a specifically designed apparatus (guillotine).

**Advantages**
- guillotines are commercially available
- tissues and blood are not contaminated by chemicals
- it is rapidly accomplished

**Disadvantages**
- aesthetically displeasing
- some studies indicate that the animal may not lose consciousness for 13-14 seconds after decapitation
- handling and restraint required may be distressful to the animal
- Inherent risk of injury to personnel

**Recommendation**
This method is acceptable with conditions if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IA-CUC. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

**Exsanguination Under Anesthesia**
Exsanguination is acceptable in all species, but because of the anxiety associated with extreme hypovolemia, exsanguination may only be done in sedated or anesthetized animals.

**CHEMICAL AGENTS**
Intravenous administration is the most rapid and reliable method of performing euthanasia with injectable euthanasia agents. It is the most desirable method when it can be performed without causing fear or distress in the animal. Sedation of aggressive, fearful, wild, or feral animals should be accomplished prior to intravenous administration of the euthanasia agent.

When intravenous administration is considered impractical or impossible (e.g., in animals weighing 7 kg), intraperitoneal administration of a nonirritating euthanasia agent is acceptable when this approach would cause less distress than I.V. injection and provided that it does not contain neuromuscular blocking agents. Intracardiac administration is not considered acceptable in awake animals, owing to the difficulty and unpredictability of performing the injection accurately. Intracardiac injection is acceptable only when performed on heavily sedated, anesthetized, or comatose animals.

When injectable euthanasia agents are administered other than intravenously, animals may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small cages in a quiet area to minimize excitement and trauma.

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

**Advantages**
- speed of action
- anesthetically pleasing when administered by trained personnel
- may be less expensive than other agents

**Disadvantages**
- must be administered intravenously by trained personnel
- animal(s) must be restrained
- controlled substance (United States Drug Enforcement Agency)
- terminal gasp may occur

**Recommendation**
Intravenous administration of barbiturates is preferred for the rabbit. It is acceptable for the mouse, rat, hamster, and guinea pig; in these species, barbiturates may be administered intraperitoneally or intracardiac (see above) as alternate routes.

**INHALANT ANESTHETICS**
Occupational exposure to inhalation anesthetics constitutes a human health hazard. An increased incidence of spontaneous abortion and congenital abnormalities results from exposure to trace amounts of inhalation anesthetic agents. Human exposure levels for volatile liquid anesthetics (ether, halothane, methoxyflurane, ethrane, and isoflurane) should be less than 2 ppm, and less than 25 ppm for nitrous oxide. While there are not controlled studies proving that such levels of anesthetics are "safe," these concentrations were established because they were shown to be
attainable under hospital conditions. Effective procedures must be employed to protect personnel from anesthetic vapors.

**Ether, Halothane, and Methoxyflurane**
The inhalant anesthetics (ether, halothane, and methoxyflurane) will produce anesthesia and death when administered as an overdose. Mice, rats, hamsters, and guinea pigs are placed individually in a closed chamber containing cotton or gauze soaked with the anesthetic. Because the liquid anesthetic is a skin irritant, the cotton or gauze must be placed under a raised wire floor and the animals exposed to vapors only.

**Advantage**
- halothane and methoxyflurane are nonflammable under ordinary conditions

**Disadvantages**
- ether is flammable and explosive, it must be used within a chemical fume hood and carcasses must be “aired” before disposing
- struggling and anxiety may occur
- personnel and other animals need to be protected
- halothane and methoxyflurane are expensive

**Recommendation**
Chamber administration of ether, halothane, and methoxyflurane is acceptable for mice, rats, hamsters, and guinea pigs. Chamber administration of these anesthetics is recommended for small animals (<7kg) where the following contingencies can be met:
- In those species where aversion or overt escape behaviors have not been noted, exposure to high concentrations resulting in rapid loss of consciousness is preferred. Otherwise, gradual fill methods can be used, keeping in mind the effect that chamber volume, flow rate, and anesthetic concentration will have on the time constant and rate of rise of anesthetic concentration. Inhaled anesthetics can be administered as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through inhaled anesthetic agent exposure and then subsequently killed by a secondary method.
- Order of preference is isoflurane, halothane, sevoflurane, enflurane, methoxyflurane. Methoxyflurane is acceptable with conditions only if other agents or methods are not available. Ether is not acceptable for euthanasia.
- Although acceptable, inhaled anesthetics are generally not used for larger animals because of cost and difficulty of administration.
- Exposure of workers to anesthetics must comply with state and federal occupational health and safety regulations.

**Carbon Dioxide**
Inhalation of $\text{CO}_2$ causes respiratory acidosis and produces a reversible anesthetic state by rapidly decreasing intracellular pH. Both basal and evoked neural activity are depressed soon after inhalation of 100% $\text{CO}_2$. Inhalation of $\text{CO}_2$ at a concentration of 7.5% increases pain threshold, and concentrations of 30% and higher cause deep anesthesia and death with prolonged exposure.
Methods to administer CO₂ include placing animals directly into a closed, prefilled chamber containing CO₂, or exposure to a gradually increasing concentration of CO₂. Carbon dioxide has the potential to cause distress in animals via three different mechanisms: (1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of so-called air hunger and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response.

**Advantages**
- carbon dioxide will produce a narcosis with rapid anesthesia
- carbon dioxide is supplied in pressurized cylinders and as dry ice
- inexpensive
- normally not hazardous to personnel or other animals
- does not distort cellular architecture

**Disadvantages**
- chamber must be completely filled and precharged to prevent animals from climbing to avoid exposure
- immature animals require more exposure time to produce unconsciousness and death

**Recommendation**
Carbon dioxide administered to individual animals by inhalation in a chamber is acceptable for mice, rats, hamster, and guinea pigs. The chamber must be fully charged before introducing animals. Only regulated pressurized carbon dioxide in cylinders can be used.

**POSTMORTEM TISSUE EFFECTS OF EUTHANASIA**
A method of euthanasia may cause direct or indirect effects on the intravascular compartment and/or histologic/electron microscopic findings. Methods which cause anoxia depend on the rapidity of induction of the anoxic state and occur as a result of changes in blood gases. The indirect effects are a result of tissue hypoxia due to the death of the animal. CNS damage occurs most rapidly. An animal should be handled properly prior to death and the tissues processed as soon as possible.

**SUMMARY**
The use of animals in biomedical research is a privilege, not a right. That privilege places a great deal of responsibility with the supervising scientist to assure compliance with the highest scientific, regulatory, and societal values. At no time is this compliance more subject to review and scrutiny than when it becomes necessary to kill the animals that have been involved in a study. The importance of this final step is emphasized by the prominence of the issue of euthanasia in the regulations, policies, and guidelines of the various regulatory, accrediting, and funding agencies. If the “good death” definition is employed as the standard for technique evaluation, then one should be able to proceed with the confidence of carrying out the responsibility that comes with the privilege of using animals in research, teaching and testing.