Lifespan System-wide Policy	Subject:	File Under:
	Lifespan Policy for the Responsible Conduct of Animal Research and Use of Central Research Facilities	ORA CRF 001
Issuing Department: Lifespan Office of Research Administration		**Latest Revision Date: 11/19/2014**
Original Policy Date: June 14, 2011	**Page 1** **See attached text**	**Approved by:** Peggy McGill, MA. CRA Director, Office of Res. Admin Peter J. Snyder, PhD Sr. Vice President and Chief Research Officer

I. Purpose: The Purpose of this Policy and Procedure Manual is to define and describe the policies and procedures regulating the use of animals in research and the appropriate utilization of the Central Research Facilities

II. Eligibility: The entire research community of The Rhode Island Hospital (collectively known as Lifespan for the purposes of this manual)

III. Content: The Manual is attached

Contents

I. Purpose and Scope of Manual 5

II. Description of the Lifespan Facilities 5

III. Training and Orientation Procedures 6

A. Requirement 6

B. Implementation 6

C. Educational Program 7

IV. Reporting Animal Care and Use Concerns 11

V. Security and Biosafety within the CRF 12

A. Admittance to the CRF Animal Facilities 12

B. Infection Control 12

C. Autoclaves 12

D. Animal Biosafety Criteria: 13

E. Personnel Occupational Health Program (POHP) 13

1. Pre-Employment Phase 13

2. New Employee Phase 13

3. Daily Operations Phase 13

4. Volunteers and Students 14

5. Contractors and Visitors 14

F. Standard Precautions 14

G. Respiratory Protection 14

1. Conventional animal rooms 15

2. ABSL2 rooms 15

3. Yearly Fit Test 15

H. Eye protection 15

I. Precautions for Invasive Procedures: 16

J. Precautions for Laboratories: 16

K. Precautions for Personnel Working with Animals 17

L. Zoonotic Diseases 17

Disease Transmission and Prevention: 18

M. Biosafety Levels for Animal Diseases (Zoonotic Agents) 18

N. Safety Procedures for the Use of Non-Formalinized (unfixed) Animal Tissue 19

O. Use of Biohazardous or Chemically Hazardous Substances in Animal Research 20

Guidelines for Use of Hazardous Substances: 21
P. Use of Human Tissues in Laboratories 22
Q. Inactivation of Recombinant DNA Materials 22
R. Use of Radioactive Materials in Animal Research 23
S. Chemical Safety 24
T. Physical Safety 25
U. Other Accidental Injuries 26
V. Reporting Safety Concerns 27
VI. Veterinary Care 27
A. Role of Veterinary Care 27
B. Veterinary Consultative Services 28
C. Reporting of Sick or Injured Animals (Clinical Medicine) 29
D. Utilization of Anesthetics and Analgesics 30
E. Use of Controlled Substances in Animal Research 32
F. Pharmaceutical Grade Drugs 32
G. Standard Operational Procedures for Survival Surgery 33
1. Large Animal Survival Surgery 33
2. Rodent Surgery Overview 33
3. Post-Operative Care 35
H. Differentiating between Major and Minor Survival Surgery-Veterinary Perspective 36
I. Conditions for Multiple Major Survival Surgeries 38
J. Expired Medical Materials Policy 39
K. IACUC Policy for the Humane Euthanasia of Laboratory Animals 40
L. Animal Health Program 47
M. Animal Health Surveillance 47
N. Rodent Health Monitoring Program 48
Response to Positive Murine Pathogen Findings in Lifespan Facilities 48
O. IACUC Policy for Tumor Implantation 51
Utilization of transplantable tumors, cell lines and other biologics 56
P. Policy on Use of Human Source Tissues and Cells in Immunodeficient Animals 56
R. Environmental Enrichment Program for Laboratory Animals 57
S. Mouse Tail Biopsy 59
T. Rodent Toe Clipping for Biopsy and Genotyping 60

U. Separating and Weaning Rodents 61
V. Social Housing 63
VII. General Information 64
A. Animal Procurement 64
B. Conditioning Period 64
C. Animal Transfer Policy 65
D. Quarantine (Importation) Requirements 65
E. Transportation of Animals 68
1. Between Buildings on Campus: 68
2. Between Main Campus and Off Sites 69
3. Between Lifespan and Brown University Facilities 69
4. Between Institutions 69
5. Patient Areas 69
6. Miscellaneous 69
F. Per Diem and Other Billable Expenses 69
G. Identification of Animals 70
H. Husbandry 70
I. Use of Image Capturing Devices 73
J. Use of Animals in Clinical Areas- Sanitation Protocol 74
K. Policy on the Review of Animal Cadavers or Animal Parts Used in Research 75
L. Use of Non-pharmaceutical Grade Sodium Pentobarbital. 76
M. Use of Avian Embryos. 79
N. Guidelines for Counting Animals Used in Research 80

Separate appendices:

1. **ORA Organizational chart**
2. **Zooonosis of Concern in Animal Care Facilities**
3. **Selection and Use of Anesthesia and Analgesia**
4. **Guidelines for Rodent Survival Surgery**
5. **Post-Op Animal Treatment Form**
6. **Animal Health Program**
7. **Cage Card Sample**
8. **Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols**
9. **Cadaver and/or Animal Parts Form**
10. **Tumor Monitoring Form**
11. **Notice of Intent to Use Avian Embryos Form**

I. I. Purpose and Scope of Manual

The purpose of this manual is to provide researchers with an overview of responsibilities in conducting animal research at Lifespan, as well as details in procuring, housing and other aspects of animal care. In addition, we have provided details in safe working practices in the Central Research Facilities (CRF).

All research at Lifespan that involves animal subjects must be reviewed and approved in accordance with federal law and Lifespan policy. The Animal Care and Use Program at Lifespan is consistent with the *Guide for the Care and Use of Laboratory Animals* (the *Guide*), the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy) and the *Animal Welfare Act Regulations* (AWRs).

Lifespan's Animal Welfare Committee (AWC) or Institutional Animal Care and Use Committee (IACUC) is charged with overseeing compliance with these federal regulations. The goal of these regulations is to ensure the safety, respect, and dignity of animal subjects involved in scientific research, and is a cooperative effort between the IACUC, Administration, Principal Investigators (PI), laboratory staff, and animal care staff. Details regarding the Animal Care and Use Program, IACUC function, operation, and review requirements are included in the *Lifespan Institutional Animal Care and Use Committee (IACUC) Policy and Procedure Manual*, ORA RRC 002, IACUC, January 2014. See also *Appendix I- ORA Organizational Chart.*

All forms and additional guidance and informational links may be found at http://www.lifespan.org/research/administration/animal-research.html.

II. Description of the Lifespan Facilities

The Central Research Facilities (CRF) consists of 30,350 net sq. ft. in the following four functions: Central Animal Facilities (CAF); Washing/Sterilizing Facilities; Operating Rooms/Veterinary Services; and Research Operations. The CRF functions are located at Rhode Island Hospital (RIH) in the Middle, Aldrich and Nursing Arts Buildings; the Claverick Street Building; and the Coro West and Coro East Buildings.

Lifespan has an Animal Welfare Assurance on file with OLAW. The Animal Welfare Assurance number is A3922-01. The USDA license # is 15-R-0002, issued 7/31/1967.

The Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs – Lifespan's institutional accreditation by AAALAC dates to May of 1970. (The original accreditation was for The Miriam Hospital; Rhode Island Hospital first received accreditation in 1996 after the two hospitals were joined under the Lifespan parent organization in 1994). AAALAC International has continued full accreditation for Lifespan's Animal Care and Use Program under file number 205.

The facilities are monitored by a variety of security measures and entrance into the CRF is by permission only.

III. Training and Orientation Procedures

A. Requirement

The Animal Welfare Act and Public Health Service Policy requires that research facilities ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use be qualified to perform their duties. In addition the qualifications of the personnel must be reviewed frequently enough to assure continued compliance. This training and instruction must include guidance in the following:

- Humane methods of animal care and use,
- Methods to limit the use of animals or minimize animal distress,
- Proper use of anesthetics and analgesics,
- Methods to report deficiencies in animal care and treatment
- Utilization of information services, e.g., the National Library of Medicine and the National Agricultural Library.

B. Implementation

The hospital has implemented a formal educational program on animal care and use to assure compliance with these requirements. The Administrative Director of the Office of Research Administration delegates the responsibility for the implementation and the continued development of this program to the Director of the Central Research Facilities and the Attending Veterinarian. The educational program has been approved by the Institutional Animal Care and Use Committee and is reviewed semi-annually as part of its responsibility to review the Animal Care Program.

- **All personnel involved in Biomedical Research Laboratories, in any capacity, must attend the CRF orientation/training. All newly hired research investigators, personnel, volunteers and students must contact the CRF office at 444-5788 to schedule training and orientation, even if they do not work with animals.**

At the time of initial contact, the CRF user will complete a request for Laboratory Animal Procedures and Privileges (LAPP) as well as the Health Surveillance Questionnaire (HSQ). The content and delivery of the training/orientation will be determined by the CRF management.

Additional training in anesthesia, aseptic surgery techniques and the use of the operating room for chronic surgery requests must be requested by the research personnel by contacting the Operating Room Supervisor at 444-5607. Mandatory training for use of the dark rooms and autoclaving units may be arranged by contacting the CRF Office at 444-5788.

An annual training refresher is required for anyone utilizing animals. The training is customized for rodent users or large animal users. Annual Training is available on-line at CITI (Collaborative Institutional Training Initiative) www.citiprogram.org. Training completion dates are recorded on the Animal Care and Use Protocol form (ACUP) and the annual progress report forms for continuing review are verified by the IACUC Coordinator during the pre-review process.

C. Educational Program

The program is intended to assure the continued excellence in animal care and scientific investigation as well as to comply with all federal, state and local regulations concerning animal related research. Assistance and guidance are provided through various forums including: (1) an Introduction/Orientation to the Central Animal Facilities, (2) veterinary consultation with the Principal Investigators during the preparation of a new Animal Care and Use Protocol (ACUP); (3) individual or group instruction on specific animal use techniques; (4) continuing education; (5) training for new animal care technicians. Additional details concerning these forums follow.

1. **Introduction/Orientation:** All personnel using animals at Rhode Island Hospital or submitting ACUPs are required to attend an Orientation meeting at the Central Research Facilities (CRF). At that time, a PowerPoint Presentation will be given which includes an overview of the federal regulatory and accreditation agencies.

   Each person is instructed on the methods for reporting deficiencies in animal care and treatment and is provided a link to the CRF website where the CRF Policy and Procedures Manual resides. The orientation packet includes the RIH policy on humane animal care and handling, general rules and procedures in the animal facilities, reference tables for typical laboratory animal species and membership rosters for the IACUC, Biohazards and Laboratory Safety Committee and Recombinant DNA Committees. A Lab Animal Privileges and Procedures Training Documentation Form of each person's past experience with animals is completed. This form must be kept accessible in the laboratory and updated as new training is completed. After the orientation presentation, a tour of the Animal Facility is given.

2. **Preparation of a new application:** The veterinarian provides consultation to the investigators during the planning and implementation of animal use proposals, which the Principal Investigator then indicates on the ACUP application form prior to submission to the Institutional Animal Care and Use Committee. This consultation is used to advise the investigator on the selection of experimental models, including consideration of alternatives to painful procedures; give directions and recommendations for the use of anesthetics, analgesics and euthanasia methods, and the prohibition of the use of paralytics without anesthesia. The Attending Veterinarian also makes an assessment concerning the qualifications and training of the investigator and staff to provide humane care for the animals and to perform the procedures so that pain and distress will be minimized.

3. **Individual or Group Instruction:** Veterinary Services provides instruction on humane methods of animal maintenance, restraint, and experimental technique as needed or at the request of a person or laboratory. Areas of interest might be common technical procedures including various methods for giving injections, blood sampling or animal restraint. Veterinary Services provides instruction on aseptic surgical techniques or anesthesia. These personnel can be contacted through the CRF (444-5788).

4. **Continuing Education:** Information is provided to the research community through internal memos. Issues addressed would include changes in the Animal Welfare Act, NIH guidelines for the care and use of laboratory animals, publications of the National Agricultural Library or the National Medical Library, and information on animal issues

obtained at national and regional seminars. The Central Research Facilities also has numerous books and other materials which can be consulted. Members of IACUC are provided with educational materials and reprints at each monthly meeting. As noted in B above, the IACUC requires annual training for every person involved with the care and/or use of animals.

5. **Training for Animal Care Technicians:** Each technician, including volunteers, receives extensive one-on-one instruction on proper care and handling of each species housed at the hospital prior to receiving work assignments. The majority of the Animal Care Technician training is provided by Veterinary Services and CAF Supervisors. Volunteers receive their instruction while under the direct supervision of their assigned Animal Care Technician. The Attending Veterinarian and/or investigators present specific animal requirements to the staff and discuss zoonosis, radiation or toxic hazards that may be involved in animal research. All technicians are strongly encouraged to attend continuing education and seek certification by the American Association of Laboratory Animal Science.

6. **Use of Animals in Training Courses:** Instructors (or appropriate designees) of any course involving animals must attend the Central Research Facilities (CRF) Orientation. Contact Central Research Facilities (444-5788) to make arrangements for orientation.

   Students of any courses involving animals are not required to attend the orientation as long as their contact with animals is limited to procedures under the direct supervision of the instructor (or appropriate designee). Instructors (or appropriate designees) should inform all participants of the existence of the IACUC, that this course has been approved by the Committee and that anyone is welcome to discuss the hospital's animal care and use policies with the IACUC Chair, Director of CRF or Attending Veterinarian if they have any questions.

   Pre-op preparation of large animals for procedures and post-op care, if any, is the responsibility of the Research OR/Vet Services. A tutorial on rodent surgical techniques is available on-line through the AALAS Learning Library. Contact the CRF (444-5788) for access to the Library.

7. **Training Requirements for the Research Community**

Health Surveillance and Training Requirements for RIH Research/Training Course Staff

The following table provides an overview of the training required for faculty and staff involved in research or training at Lifespan (or Women & Infants) that involves the use of animals or animal tissue.

	Principal Investigators		Research Staff			CAF Staff			
	Hands-On	**Admin(1)**	**Principal Researcher**	**Research Assistants**	**Technicians**	**Vet Supervisors**	**Vet Coordinator**	**Animal Care Technicians**	**IACUC Members**
CAF Orientation for new employees	Required	Required	Required	Required	Required	Required	Required	Required	
Annual Health Surveillance	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually
CITI Training Modules									
Essentials for IACUC Members									Required
Working with the IACUC	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Encouraged	
Annual rodent training	Users(2) Annually		Users(2) Annually	Users(2) Annually	Users(2) Annually	Required Annually	Required Annually	Required Annually	
Annual large animal training	Users(2) Annually		Users(2) Annually	Users(2) Annually	Users(2) Annually	Required Annually	Required Annually	Required Annually	
Working with Mice in Research									
Working with Rats in Research									
Working with Rabbits in									

Research									
Working with Swine in Research									
Working with Zebra Fish in Research	Users(2) required at initial review		Users(2) required at initial review	Users(2) required at initial review	Users(2) required at initial review				
Post-Procedure Care of Mice & Rats									
AAALAS Learning Library (4)									
Rodent Surgery	Users(2) required at initial review		Users(2) required at initial review	Users(2) required at initial review	Users(2) required at initial review	Required	Required	Encouraged	
Hands-On Procedural Training									
All procedures performed independently (documented on training form)	Required (3) at initial review		Required (3) at initial review	Required (3) at initial review	Required (3) at initial review	Required (3) at initial review			

Notes:

1) Administrative PIs are those who direct research or training programs, but who are not personally involved in working with animals.

2) Users are defined as anyone who is involved in direct hands-on use of animals, or supervising/training of others who are.

3) Technical proficiency must be documented on the Lifespan Animal Privileges and Procedures Training Form via signature by an expert assessor before procedures may be performed independently on live animals. Expert assessors include anyone with documented proficiency in the procedure, such as a more senior lab member (e.g. PI, senior researcher, lab manager and technician), a CAF staff member, or one of the attending veterinarians.

4) The AALAS Library is a subscription service. Contact the CRF Main Office at 444-5788 to gain access to the library.

The Central Research Facilities Office (444-5788) can be contacted for information or assistance concerning the care and use of animals or for specific technical needs.

IV. Reporting Animal Care and Use Concerns

Individuals having concerns involving animal care and use within Lifespan facilities are responsible for reporting these concerns either through their supervisor or independently to the IACUC and can be made through various persons, e.g., any member of the IACUC, IACUC Coordinator, Director of CRF, CRF supervisors, veterinarians, the Institutional Official (Sr. Vice President & Chief Research Officer), the Administrative Director of Research Administration, or the Director of the Research Protection Office verbally or in writing. IACUC contact information is posted on the IACUC webpage as well as provided to all researchers during their initial orientation with the Central Research Facilities (CRF) Director. Veterinary and CRF management staff telephone numbers are posted within each animal facility. Alternatively reports may be submitted anonymously to Corporate Compliance via the Employee Response Line at 888-678-5111.

Although written concerns are more convenient to handle, complainants may not be willing to submit them in this manner. In such cases, the individuals who receive concerns should document them fully to ensure that the issues are clear and to prevent misunderstandings.

Lifespan will take appropriate steps to protect the confidentiality of those who report concerns as well as anyone against whom allegations are directed, while allegations are under investigation.

Lifespan policy prohibits unlawful retaliation against employees as a consequence of good faith actions in the reporting or the participation in an investigation pertaining to allegations of wrongdoing.

See Lifespan Institutional Animal Care and Use Committee (IACUC) Policy and Procedure Manual, ORA RRC 002, IACUC, January 2014, Section 8.

V. Security and Biosafety within the CRF

A. Admittance to the CRF Animal Facilities

The Central Animal Facilities (CAF) are restricted areas and secured at all times. Only personnel authorized by the CRF Director are permitted into the animal facilities. No one will be given access to the CAF until mandatory training is completed and documented. All keys and access materials must be returned to the CRF office upon termination from Lifespan.

Animals may be transported to and from the CAF under an approved IACUC protocol, but under no circumstances are animals to be housed outside the CAF overnight.

B. Infection Control

All persons using the facilities are required to follow the Hospital Infection Control Manual and Standard Precautions.

All employees having contact with human blood and body fluids are encouraged to receive Hepatitis B vaccine.

All employees working with animals must have a full primary series of tetanus and a booster (Td/Tdap) every 10 years. Rabies vaccination is available but is not required.

All persons using the facilities must be oriented to Infection Control and Standard Precautions as described in the hospital Infection Control Manual. All orientation records and updates must be documented.

C. Autoclaves

The sterilization process monitoring includes the function of the sterilizer, type and method of packaging and the loading of the sterilizer. Sterilizers are monitored with a biological spore test weekly and records of the monitoring are maintained. All persons responsible for use of sterilizers must be oriented to the proper use of sterilizers and that orientation must be documented. The CRF is responsible to see that the sterilizers are monitored, and that education is documented.

1. A log must be kept by each autoclave with the names of every user as well as their instructors.
2. Every load must have a steam indicator and a steam load record card.
3. Once a week, a spore test must be run with a normal load. The spore test pack is sent to the Veterinary Services office. The spore test pack is placed in an incubator for the appropriate process time.
4. The spore test results are logged by Veterinary Services and sent quarterly to the Infection Control Department along with preventative maintenance service reports. These reports are kept on file in the Veterinary Services office.
5. The CRF has established a maintenance service agreement. Preventive maintenance is performed regularly on all sterilizers. All preventive maintenance documentation is on file in the CRF. A log is kept for the service visits.

D. Animal Biosafety Criteria:

The hospital safety officers are charged with enforcing biosafety guidelines. In general, investigators are required to follow the recommendations presented in Section IV of the *Biosafety in Microbiological and Biomedical Laboratories Manual*, published by the Centers for Disease Control and The National Institutes of Health. *http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf*. These recommendations describe four combinations of practices, safety equipment, and facilities for experiments on animals infected with agents, which are known to or believed to produce infections in humans. These four combinations, designated Animal Biosafety levels 1-4, describe animal facilities and practices applicable to work on animals infected with agent assigned to corresponding biosafety levels 1-4. The high confinement requirements for Animal Biosafety levels 3 and 4 cannot be met at any of the RIH facilities.

E. Personnel Occupational Health Program (POHP)

Personnel hired to work in the Central Research Facilities (CRF), or any biomedical research area, are given pre-employment physical examinations by Employee & Occupational Health Services (EOHS).

1. Pre-Employment Phase

Each job applicant for a CRF or biomedical research laboratory position will receive the standard pre-employment medical examination at the Employee & Occupational Health Services (EOHS). In addition, the following examinations may also occur: history for allergies especially to animals and animal by-products, a history of orthopedic problems, e.g. bad backs, knees and problems preventing lifting, carrying, reaching and stretching in job context, and medical evaluation for ability to wear respirator masks.

2. New Employee Phase

Before assignment to animal care duties, all new biomedical lab personnel will be immunized against tetanus (or provide written evidence of recent immunization or booster), offered the opportunity for immunization against rabies, scheduled for a hospital orientation, receive departmental training and will attend the Radiation Safety course offered by the Radiation Safety Officer, as soon as it is offered.

3. Daily Operations Phase

a. Personnel showing signs of non-work related illness during the work day may be referred to EOHS for treatment. Clearance from the EOHS is required before the technician can return to work.

b. Job injuries or illness recognized or otherwise occurring during the work day, including all animal bites and scratches, will be reported immediately to the laboratory supervisor and referred promptly to the EOHS. A formal detailed record of diagnoses and treatment activity will be maintained by the EOHS of each incident. Clearance from the EOHS is required before the technician can return to work. A copy of each incident report will be sent to the Lab Supervisor.

4. Volunteers and Students

Volunteers / students working in a research laboratory with protocols using animals are also covered by the POHP. Volunteers/students receive an interview at the time of CRF orientation to assess history for allergies, especially to animals and animal by-product, history of orthopedic problems, i.e. bad back, knees and problems relating to lifting, carrying, reaching and stretching, and evaluation for ability to wear masks.

One or more of the following services will be provided or offered according to the area and the animals the volunteer / student will be working with: health screening, hepatitis B vaccine, TB surveillance, tetanus toxoid.

5. Contractors and Visitors

Non-RIH/Lifespan contractors and visitors entering the CAF must follow all PPE requirements. If a respirator is needed, that person must be cleared by their employer's occupational health program.

F. Standard Precautions

Standard Precautions includes the following elements and must be followed by **ALL PERSONNEL AT ALL TIMES**. These precautions apply to contaminated medical equipment. Body substances included in standard precautions are: blood (human and animal), urine, stool, oral secretions, wound and tissue. The precautions take into consideration the degree and risk of exposure. Appropriate judgment must be used in determining the protective measures needed for maximum protection.

1. Wear gloves whenever hands will be in contact with blood or body substance (blood, urine, stool, oral secretions, wound or other drainage, or tissue). This includes all contact with animals or soiled animal equipment. Discard gloves and perform hand hygiene.
2. In the event of an accidental skin exposure, hands or other exposed areas must be washed with soap and water as soon as possible.
3. Care must be taken to avoid needle stick injuries. Used needles must not be recapped or bent, but must be placed in the puncture resistant containers designed for such disposal.
4. Report significant exposure (needle sticks, mucous membrane splash) to EOHS for evaluation and follow-up.

G. Respiratory Protection

The primary objective is to prevent potential occupational exposures caused by the inhalation of contaminated air. Central Research Facilities will attempt to accomplish this by accepted engineering control measures and practices (e.g., biosafety cabinets, changing and dumping stations). When effective engineering controls are not feasible or practical, appropriate respirators shall be used.

Respirators which are suitable for the intended purpose shall be provided to all employees. N95 respirators will be provided by Central Research Facilities when such equipment is necessary to protect the health of the employee. Full or half face respirators, or PAPR (powered air purifying respirator) will be provided by the employee's department. Central Research Facilities shall adhere to the Hospital's Respiratory Protection Program (Environmental Safety Department, policy SM-15) which includes the requirements as outlined within OSHA 29 CFR 1910.134, *et al*.

The procedures for the use and maintenance of respirators for employees while conducting their normal animal care work-duties and instructions on selecting the appropriate respirator for each specific function~~s~~ or area/room are described below. There may be additional requirements depending on the hazard or potential exposure. In such cases, Central Research Facilities Management in conjunction with the Safety Department will determine the appropriate respiratory protection in accordance with the OSHA Standards.

Animal care technicians, investigators, laboratory personnel, and CRF management staff are to don air purifying respirators depending upon work/room functions. When donning the chosen air purifying respirator, a user seal check (i.e., fit check) must be conducted prior to entering the work area.

1. **Conventional animal rooms**

   A number of engineering controls will be implemented to limit exposure to contaminated air. These will include the increased use of ventilated cages, microisolator covers for cages, use of fan driven, HEPA filtered environmentally isolated caging units, use of portable changing stations for changing cages, and use of filtered dumping stations/hoods for dumping cages. N95 particulate respirators must be donned while dumping cages if dumping stations/hoods are not available for use. Once the dirty cages are in the washer, animal care personnel may remove their respirator.

2. **ABSL2 rooms**

   In ABSL2 rooms, personnel are to don N95 respirators for any procedure being done in these rooms including checking cages, cage changing, opening cages, or handling the cages or animals for any reason. Each protocol requiring animals to be housed in ABSL2 rooms will be evaluated for respirator usage by the Biohazard and Laboratory Safety Committee. They may deem that full face respirators be used for handling animals that are part of certain protocols.

3. **Yearly Fit Test**

   All personnel that wear respirators (N95 particulate, full face or other respirators) are to be fit tested yearly by the Hospital Safety Office. If personnel have problems wearing indicated respirators, there may be alternative respirator types/styles that may be more suitable/comfortable. In such instances, personnel should report to their supervisor who will coordinate with the Safety Office for appropriate recommendations.

H. Eye protection

Eye protection (safety glasses, chemical-resistant goggles, or face shield) must be worn at all times in the animal facility when handling chemicals, including detergents, disinfectants and/or hazardous materials. Use the appropriate eye protection for the kind of hazard in the work area. Ordinary prescription glasses are not considered effective eye protection since they lack necessary shielding. Safety glasses with side shields offer minimal protection; splash goggles and face shields offer greater protection for procedures involving liquids. Chemical-resistant goggles can be worn over the glasses. Safety glasses or chemical-resistant goggles shall be worn over contact lenses when handling chemicals. Safety glasses protect from impact. Goggles protect against impact, dust, and splashes. Face shields are generally worn over safety glasses or goggles to protect the face from dusts, sprays or splashes. Only equipment certified by the American National Standards Institute qualifies as

protective eyewear. The safety office encourages laboratory personnel to wear eye protection at all times when in a laboratory.

I. Precautions for Invasive Procedures:

The standard precautions listed above in Section F, combined with those listed below, should be the minimum precautions for ALL invasive procedures.

All workers who participate in invasive procedures must routinely use appropriate barrier precautions to prevent skin and mucous membrane contact with blood and other body fluids. Gloves and surgical masks must be worn for ALL invasive procedures.

Protective eye wear or face shields should be worn for procedures that commonly result in the generation of droplets, splashing of blood or other body fluids, or the generation of bone chips.

Gowns or aprons made of materials that provide an effective barrier should be worn during invasive procedures that are likely to result in the splashing of blood or other body fluids.

J. Precautions for Laboratories:

The Standard Precautions listed above in Section F, combined with those listed below, should be the minimum precautions for workers in laboratories.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.

2. All persons processing blood and body fluid specimens (e.g., removing tops from vacuum tubes) should wear gloves. Masks and protective eye wear should be worn if mucous membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.

3. For routine procedures, such as histologic and pathologic studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets (Class I or II) should be used whenever procedures are conducted that have a high potential for generating droplets from open containers. These include activities such as blending, sonicating, and vigorous mixing.

4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.

5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under Standard Precautions should be followed.

6. Laboratory work surfaces must be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.

7. Contaminated materials used in laboratory tests must be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.

8. All persons should perform hand hygiene after completing laboratory activities and should remove protective clothing before leaving the laboratory.

Implementation of Standard Precautions for all specimens eliminates the need for warning labels on specimens since all specimens should be considered infectious.

K. Precautions for Personnel Working with Animals

Personnel working with animal subjects must maintain high standards of personal hygiene. Though rare, transmission of disease between animal and man has been clearly documented.

1. All personnel should wear sterile or disposable gowns, scrubs and gloves, or other appropriate apparel when working with animals. Laboratory apparel should be changed frequently to maintain cleanliness and minimize the potential for cross contamination between animals and between rooms.
2. All personnel should sanitize their hands thoroughly before entering and upon leaving an animal room to insure personal protection and to minimize any potential for cross contamination between animals and rooms.
3. Dispose of broken glass, needles, and other sharp hazards in proper containers.
4. Eating, drinking, and smoking are not permitted in the animal facility. Food and drink may only be consumed in the CRF offices and staff room.
5. Pets are not allowed into any of the animal care facilities under any circumstances.

L. Zoonotic Diseases

A zoonotic disease, or zoonosis, is an infectious disease which can be transmitted between humans and animals. Of the hundreds of zoonotic diseases known, only a handful are of concern in the research animal facility. Modern animal production techniques and animal facility operating procedures are designed to minimize the threat of zoonotic diseases, both to personnel and valuable animal colonies. When human infection does occur, it often is the result of failure to follow accepted procedures.

Prevention and control of disease in a research facility includes vendor selection, animal receiving, quarantine, facility design, animal housing, personnel traffic patterns, sanitation practices, vermin control, veterinary care and necropsy.

A list of common zoonoses of laboratory animals can be found in *Appendix 2 Zoonosis of Concern in Animal Care Facilities.*

Consult with a physician knowledgeable about animal-related diseases if you have any medical condition that may make you more susceptible to certain animal-related diseases. Such medical conditions include but are not limited to splenectomy, alcoholism, immune system problems (e.g. AIDS, chemotherapy, systemic steroids such as cortisone, cancer), tuberculosis, pregnancy, or a history of heart disease or heart surgery (even though you may not have any heart symptoms now). If your personal physician is unfamiliar with animal diseases, have him or her contact a Lifespan Employee Health physician or the Lifespan Attending Veterinarian for additional information.

Women who are pregnant can work in animal facilities, but certain tasks may present a hazard to the unborn fetus. Women who become pregnant should notify their

instructor/supervisor. The employee should consult with a Lifespan Employee & Occupational Health Services physician to review their duties while pregnant. Toxoplasmosis is a disease acquired from cats that if acquired by a pregnant woman during pregnancy can cause birth defects and other disorders in an unborn fetus. It is recommended that pregnant women not work with cats in a laboratory or farm setting.

Disease Transmission and Prevention:

1. **Presence of the Zoonotic Agent in the Animal**
   The first consideration in control of zoonotic disease is the presence of a potential disease-producing agent in the animals. Zoonoses are most effectively avoided by purchasing animals which do not harbor these agents. Most rodent vendors supply information on the disease status of animals shipped from their production facilities.
2. **Escape of the Zoonotic Agent from the Animal**
   Natural routes by which zoonotic agents are shed from animals include saliva, feces, urine, exudative skin lesions, and vectors such as biting insects. Surgery, biopsy, necropsy and removal of any animal tissue (including blood) can serve as a means of transmission.
3. **Transmission of the Zoonotic Agent to a Human**
   Direct contact with animals or animal products is the primary method of disease transmission. Recommendations for avoiding this route of disease transmission include wearing gloves and washing hands. Puncture wounds inflicted with needles used on animals are also common sources of infection. Aerosol transmission of disease-producing organisms can be minimized by wearing a face mask when working with animals or animal products.
4. **Zoonotic Agent Enters Human Host**
   Zoonotic agents can utilize four routes of entry into the human host: ingestion, inhalation, parenteral inoculation, and contact with mucous membranes (e.g. eyes or mouth). Gloves, masks, and in some cases splash-proof eye protection are used to prevent entry of zoonotic agents into humans. Hands are washed before and after handling animals or animal products. No eating, drinking, or smoking is allowed in the animal or treatment rooms. Needles must be disposed of in a puncture-proof container.
5. **Human Host Contracts Disease**
   The susceptibility of the human host for disease is dependent on a number of factors. One of the most important of these factors is the status of the host's immune system. Manipulation of the immune system through vaccination is used in some instances where potential for zoonotic disease is great. Vaccines developed for some of the zoonotic diseases are available to personnel with high risk of exposure. Tetanus and Rabies are the only diseases for which CRF personnel are currently offered vaccination.

M. Biosafety Levels for Animal Diseases (Zoonotic Agents)

Criteria and practices for zoonotic agents are based on recommendations found in Section IV of the CDC's manual, "Biosafety in Microbiological and Biomedical Laboratories" *http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf*.
Animals suspected or known to carry a zoonotic agent are assigned to a particular biosafety level. The standards of practice found in the CDC manual will be instituted by the CRF

indefinitely or until the animal is determined to be free of the particular agent. The CDC manual describes Animal Biosafety Levels 1 – 4 but only Animal Biosafety Levels 1 and 2 are allowed in the CRF program and at the CAF sites.

- **Animal Biosafety Level 1 - no risk**
  Animal diseases listed in Biosafety Level 1 are considered species-specific and as such do not fit the definition of zoonoses. These agents are not associated with disease in healthy adult humans. However, animals with diseases in this category may be banned from the CRF to prevent spread of infection to animals under study.
  Examples: mouse hepatitis, mouse pox, rat parvovirus, rabbit pox

- **Animal Biosafety Level 2 - moderate risk**
  Animal diseases listed in Biosafety Level 2 include most infectious zoonotic agents. The primary hazards of these diseases are associated with parenteral inoculation or mucous membrane exposure. Aerosols are not a common means of exposure to agents in this class. Infections with toxoplasma and herpesvirus are usually asymptomatic and often are extremely prevalent in the host species. For this reason, these animals are considered infected and handled as such.
  Examples: salmonellosis, toxoplasmosis, ringworm, leptospirosis, shigellosis
  Immunodeficient animals carrying human source tissues and tumors are also considered ABSL-2.

N. Safety Procedures for the Use of Non-Formalinized (unfixed) Animal Tissue

Humans can contract potentially serious zoonotic diseases after being exposed to non-formalinized animal tissue just as they can after exposure to live animals. Non-formalinized animal tissue originating off campus can also be a source of infection for the laboratory animals at Rhode Island Hospital. Precautions must be taken to protect hospital employees and patients from possible exposure to the more pathogenic zoonotic disease organisms and to protect the integrity of our research animal populations.

1. **Containers for Transportation**
   Non-formalinized animal tissue being transported to the Hospital or from one area to another area within the Hospital must be transported in a sealable container that can be autoclaved.

2. **Hood Requirement**
   A Class I or II hood (a biological Safety cabinet with HEPA-filtered recirculated mass airflow within the work space plus HEPA-filtered exhaust air) is required while utilizing non-formalinized animal tissue from ruminant livestock species (sheep, goats, cattle) and from non-human primates.

3. **Protective Equipment**
   Protective equipment such as disposable masks, gowns, safety glasses and gloves that are appropriate for handling potentially infectious material must be worn when working with non-formalinized tissue from ruminant livestock species and non-human primates. Lab coats and gloves are appropriate when working with other animal tissue.

4. **Decontamination/Disinfection**
   After each use, wash surfaces with Clidox-S or fresh or stable 10% bleach solution.

5. **Waste Disposal**
   Autoclave the waste before leaving it for disposal.

6. **Use in Central Animal Facilities**

   a. Non-formalinized animal tissue must not be brought into any of the Lifespan animal facilities without permission by the Veterinary Services staff. The origin of tissues must be identified.
   b. If such tissue is to be brought to the Research Operating Room in the Aldrich Building, it must be in a sealed, covered container while in transit The route would be up the Aldrich elevator and then through to the Research Operating Room. The Central Research Facilities Office (444-5788) must be notified in advance when non-formalinized animal tissue will be brought to the Research Operating Room.

7. **Responsibilities and Compliance**

   In the interest of the safety of employees, patients and the animal population, research personnel will be responsible for compliance in their area with the above procedures. Reports of noncompliance will be brought to the attention of their supervisor and the Director, CRF and the Administrative Director, Research Administration.

O. Use of Biohazardous or Chemically Hazardous Substances in Animal Research

Lifespan's Policy is to inform personnel of potential health hazards in the work place (Right to Know Act, http://www.epa.gov/emergencies/content/lawsregs/epcraover.htm). Before using any potentially hazardous substance or procedure, a detailed set of Standard Operating Procedures (SOP) for that substance or procedure needs to be written and provided to staff members. Prior approval by the Biohazards and Laboratory Safety Committee is required for:

- Chemical agents that have been assigned a safety rating of 3 or greater in any category on the MSDS sheet
- Any compound listed as a carcinogen, mutagen or teratogen in the Chemical Hygiene Plan
- Any toxin including such proteins as ricin, cholera toxin and bacterial toxins
- Any organism included in the list of Risk Group 2 (RG2) and Risk Group 3 (RG3) organisms in appendix B of the NIH Guidelines For Research Involving Recombinant or Synthetic Nucleic Acid Molecules for Research Involving Recombinant or Synthetic Nucleic Acid Molecules or organisms that require Biosafety Level Containment Level 2 or greater (BSL2, BSL3, BSL4) as defined by the Centers for Disease Control (CDC) manual Biosafety in Microbiological and Biomedical Laboratories (BMBL)
- Any organism that will be administered to live animals. [note, separate IACUC submission for any work using animals is also required]

To insure that all involved personnel are fully informed, investigators, technicians, students/volunteers, and CRF personnel will attend a mandatory training session and will comply with the SOP. This training must be documented and records maintained by the CRF office.

Note: If materials used in animal research are radioactive as well as hazardous, additional safety measures must be taken (see section on Use of Radioactive Materials in Animal Research).

Implementation of appropriate safety precautions is required by the CRF before investigators initiate studies employing hazardous substances in animal research. Investigators should be thoroughly aware of the disposition of the hazardous substance and/or its metabolites in designing the appropriate safety protocols. These precautions should maximize the safety of personnel exposed to substances either known to be or suspected of being hazardous. Consultation is available from the Biohazard Laboratory Safety Committee (BLSC), veterinary staff, the CRF staff, and the Hospital's Safety and Radiation offices.

Protocols are reviewed with respect to the use of hazardous materials as part of the protocol review process. CRF policy is based on the recommendations of the NIH, NCI, OSHA, and other federal and state regulations. The following guidelines should be incorporated into protocols involving hazardous substances.

Guidelines for Use of Hazardous Substances:

1. Use of hazardous substances in the CRF requires prior approval from the Biohazard and Laboratory Safety Committee (BLSC). These substances will only be allowed in rooms clearly identified with a hazard label. Room doors and cages must be clearly marked with the type of hazard involved, name(s) and telephone numbers of responsible investigators, and appropriates precautions to be followed.
2. Personnel working with hazardous substances, including CRF personnel, must be identified and thoroughly trained about the relevant safety precautions, potential hazards, and procedures for decontamination.
3. Protective apparel must be worn when working with hazardous substances and may include a disposable gown, N95 or other suitable respirator, eye goggles, respirator, double disposable gloves, head cover, double shoe covers, among others. The characteristics of the particular substance should be considered in selecting appropriate protective apparel.
4. Biological safety cabinets must be used when activities have a high potential for creating aerosols: intranasal inoculations, necropsy of infected animals, dumping of contaminated bedding, and manipulation of large volumes of materials. The class of safety cabinet used must reflect the risk level of the hazardous agent and/or operations.
5. Animals being used in hazard protocols must be housed in caging that confines the feed, feces, urine, and bedding in the enclosure. Cages with filter tops or HEPA-filter ventilated racks with forced air circulation are examples of such caging systems.
6. Investigators will be responsible for changing cages housing animals in hazard protocols, unless other arrangements are made with the CRF office. Certain protocols may require a laminar flow, HEPA filtered, bedding disposal cabinet.
7. All materials contaminated or in contact with hazardous substances must be decontaminated and disposed of properly. Procedures may include autoclaving, incineration, and precautions for chemical and physical cleaning. All materials to be disposed of should be double bagged in red hazard labeled bags.

For detailed information, see *Appendix 8 Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols*.

P. Use of Human Tissues in Laboratories

In the pursuit of medical training and research it may be necessary to utilize donated cadaver parts. In all cases, the academic community will treat these (parts) with respect and diligence in gratitude for their donation and strive to achieve the highest level possible of medical science and research.

Any research activity at Rhode Island Hospital utilizing human body parts must be approved by the Biohazards and Laboratory Safety Committee before starting or, if the body parts are to be received from off campus, before transportation to the Hospital.

To receive approval, the researcher must file a research application with the Biohazards and Laboratory Safety Committee. The application must include a description of the activity, certification of origin, how to be transported, where to be stored, the facility or location where the proposed research/educational activity will take place and means of disposition/disposal. The Committee may endorse or may make implementation contingent upon compliance with some recommendations.

The following SOP (Standard Operating Procedure) will be followed for embalmed and unembalmed body parts:

1. Obtain specific shipping instructions from the source prior to the shipping date.
2. Transportation shall be in a sealed autoclavable container.
3. Standard (Universal) Precautions are to be followed at all times.
4. Disposition/Disposal will be per instructions from the source which may include returning to the source of origin. Lacking instructions, the State of Rhode Island Regulated Medical Waste Rules and Regulations will be followed.
5. Supplier of body parts will describe any and all infectious disease screenings that are performed on their products.

Q. Inactivation of Recombinant DNA Materials

As specified in the NIH Guidelines for Research Involving Recombinant DNA Molecules, liquid and solid waste generated in recombinant DNA work must be decontaminated before disposal. Decontamination will be carried out by bleach treatment or steam autoclaving as appropriate. For example, bench tops and spills are best treated with bleach while culture plates, used pipettes and tubes will be autoclaved. Because of variables affecting the effectiveness of autoclave steam inactivation, the following protocol has been adopted by the RIH Recombinant DNA Committee (RIH-RDC).

All prior RIH guidelines pertaining to use of autoclaves must be followed, including logging of loads, use of indicator strips, schedule of testing, training for specific autoclaves, etc. The autoclave used for sterilization of animal facility supplies by CRF in the Nursing Arts Building is not available for waste inactivation.

Recombinant DNA waste materials must be clearly labeled. Transport to the autoclave must be in leak proof outer containers and resting in a tray or bucket inside the autoclave. Avoid transport through patient areas. Arrange materials so that steam circulates freely around them. No more than 15 pounds of material (less than 200 culture plates) may be autoclaved in one load.

For bagged dry materials, (e.g. empty pipettes), add 100-200 ml of water to the bag to generate steam and leave the bag top open inside the autoclave.

Set time for 45 minutes after reaching the temperature of 250^{o} F. This is more than necessary to kill bacteria but is the time needed to insure complete activation depending on load variations. Check that the settings are correct. Use care to avoid burns from hot liquids when removing items from the autoclave.

R. Use of Radioactive Materials in Animal Research

Investigators planning to use radioactive materials in animal subjects must submit an application to the Radiation Safety Office for authorization by the Radiation Safety Committee prior to submission of the application to the IACUC for approval. Investigators are responsible for the safety of all personnel associated with any project. Consultation is available from the Radiation Safety Officer and/or the CRF and veterinary personnel.

1. Responsibilities of the Investigator

a. Must obtain approval from the CRF Director to ensure that appropriate facilities are available for the housing of animals and/or experiment.

b. Must obtain approval of experimental protocol(s) by the appropriate RIH Committees, (e.g. Radiation Safety Committee and the Animal Welfare Committee).

c. Must advise all appropriate CAF and laboratory personnel of the nature and potential health risk of the radiation hazard to be used in the experiments.

d. Must keep detailed chronologic record of all experiments which includes numbers of animals used, use of radioisotopes (material, amount and route of administration) and the date and method of sacrifice of animals at the end of an experiment.

e. The daily animal care during the use of radioisotopes will be the responsibility of the investigator and/or designated laboratory personnel and will include the following duties:

- Placement of appropriate signs indicating the nature of the hazard on door(s) to the room(s) where animals are housed during experimentation, as well as in the cage(s) where animals are kept.
- Labeling of the waste cans in the experiment room with signs which indicate the nature of the hazard and the appropriate procedures established for the particular hazardous agent being used.
- Recommend and provide the appropriate protective clothing, gloves, and/or masks by personnel working with the animals and/or biohazard agent.
- Providing daily food and water to the animals.
- Providing change or changes of bedding and cage washing at intervals established by the CAF.
- Recording on cage cards the date of death or euthanasia of animals along with the initials of the person making the record.

- Disposal of waste material and animal carcasses according to procedure guidelines established by the appropriate review committee.

f. Any deviations from the above duties must receive authorized approval from the appropriate review committee, radiation safety office and/or the CRF Management.

2. Responsibilities of the CAF

a. Will provide equipment (if available) for animal housing.

b. Will supply feed and bedding for maintaining animals during the experimentation period. At the end of the experiment, excess feed and bedding must be disposed of and not returned to the CAF stores.

c. Will provide cage cards for project and animal identification.

d. Daily census and check of animals well being will be made by the CAF supervisor or designated person at scheduled time of day. At this time, cage cards from cages where animals died or were euthanized will be collected.

3. Radiation Safety Office Responsibilities

a. Provide information on the appropriate protective clothing, personnel monitoring, procedures and safe working times, etc., if required.

b. Regular monitoring of room for contamination outside of containment areas (i.e., waste containers and cages).

S. Chemical Safety

1. Disinfectants

The concentrated forms of some of the disinfectants used in the CRF are caustic to the skin and are only handled by CRF personnel. Only diluted working concentrations of disinfectants are provided to non-CRF personnel. Gloves are worn when handling and diluting bottles of concentrated disinfectants. If skin comes in direct contact with these chemicals, the area is immediately flushed with water for two minutes, the CRF office is informed of the incident, an incident report filed, and a visit made to EOHS by the affected personnel.

2. Detergents and cleaning solutions

Detergents and other solutions used in cage washing are supplied in concentrated forms that may cause skin irritation. CRF personnel must wear gloves when dealing with these chemicals; rinsing with copious amounts of water is indicated in case of direct skin contact. The incident must be reported to the CRF office and the EOHS office and an incident report filed.

3. Pesticides and Pest Control

CRF, in cooperation with the Environmental Services Department (ESD) at the main campus, and the Property Management Department at Coro and Claverick, has a pest control program. Every two to four weeks the facilities are inspected by a commercial pest control company,

Pesticides are not routinely used in the CRF as their presence may introduce unwanted variables in animal research. Whenever possible, pest control is by means of sanitation and/or mechanical devices. If chemical pest control is required, all investigators affected

will be consulted about any proposed treatments. Trained personnel from a pest control agency, which meets the requirements for animal and human safety, will apply pesticides. Such treatments will be performed only with authorization from the CRF Management, the attending veterinarian, and the investigators involved.

4. Anesthetics

Isoflurane is a nonflammable volatile liquid used for animal anesthesia. This agent is typically used in a precision vaporizer, where waste gases are absorbed in an activated charcoal filter or scavenged to the outside of the building via a vacuum line. For some small animal procedures, isoflurane may be used in a closed jar in a fume hood which will exhaust the waste gas.

Because of the explosive potential as well as the flammable properties of ethers and because of non-ideal anesthetic properties, their storage and use within the animal facility is PROHIBITED, except for cases in which special permission has been granted for laboratory use only. Departments which feel they need the ether for a particular use must submit a request in writing for review by the Biohazards and Laboratory Safety Committee. Such requests will also be forwarded to the Hospital's Safety Manager who serves as Chairman of the Environment of Care Committee (EOC).

Parenteral (injected) anesthetics used in the CRF generally do not pose safety hazards when properly used. Adequate animal restraint is required for animal injections. Accidental inoculation of a human with any animal anesthetic must be reported immediately to the CRF office. Medical treatment is required because many parenteral anesthetics are highly alkaline. Left untreated, tissue necrosis can occur. ***All used needles and syringes must be disposed of in sharps containers.***

T. Physical Safety

All of the accidental injuries listed below must be reported immediately to the CRF office and the EOHS office. The initial report is filed by the CAF Supervisor while EOHS documents the accident and ensures that further treatment for problems related to the accident will be provided. Reporting to the CAF Supervisor's office ensures that any hazardous situation can be corrected immediately.

1. Wounds Inflicted by Animals-Bites

Animal bites can cause severe mechanical damage and, in some instances, pose a serious threat due to disease transmission.

Prevention of animal bites is based on knowing and practicing good animal handling techniques. Familiarity with the animals and their behaviors is helpful, but unpredictable events will occur regardless of past experience. Animals exhibiting aggressive behavior should be reported at once to CAF personnel. Do not attempt to handle these animals without assistance.

The steps to take immediately following an animal bite are dependent upon the situation. The site of injury should be washed with soap and water, except in cases where the wound is severe and accompanied by extensive bleeding. Sites of bleeding should be wrapped in clean cloth and pressure should be applied to control bleeding. If necessary, allow someone to assist you in obtaining medical treatment at EOHS. Describe the

incident to the CAF Supervisor and indicate the condition, location, and status of the animal in question.

2. Wounds Inflicted by Animals-Scratches

Animals most likely to inflict scratches are cats and rabbits. A zoonotic disease, "cat scratch fever," is caused by a bacterium described in the section on *Zoonoses*. There is no known zoonotic disease associated with rabbit scratches, but the mechanical damage caused by the hind claws of a rabbit can be extensive.

Proper techniques for handling animals will prevent the infliction of most scratches. It is important to realize that scratching is a rabbit's primary defense mechanism when cornered or frightened. The techniques for handling rabbits are devised to prevent scratching while providing adequate support for the rabbit's back. Animals exhibiting aggressive behavior should be reported to CAF personnel. Do not attempt to handle aggressive animals without assistance.

Actions to take if you are scratched by an animal are the same as those to be taken following an animal bite (see previous section). Be sure to notify the CAF Supervisor and obtain medical attention.

U. Other Accidental Injuries

The CAF poses many of the same hazards as any general laboratory. Accidental injuries due to safety problems such as those described below should be treated immediately in accordance with general first aid principles. Report the accident to a CAF supervisor and obtain medical attention at EOHS. The initial report filed with EOHS documents the accident and ensures that further treatment for problems related to the accident will be provided.

1. Burns

Steam released from equipment used in cleaning and disinfecting procedures is a major hazard. Only personnel who have been trained in the use of these items should handle autoclaves, cage washing machines, and portable steam cleaning units. Periodic inspection and maintenance are required to ensure that equipment is in proper working condition.

2. Falls

The major cause of falls in the CAF is water on the floor. Wet floors are common in all areas due to necessary mopping and sanitation procedures. This hazard is prevalent in cage washing areas and animal rooms, which are cleaned with large quantities of water.

3. Skin Lacerations or Punctures

Many of the materials in the CAF have the potential for causing laceration or puncture **of** the skin. Skin trauma can lead to a variety of local and systemic infections**.** Tetanus prophylaxis is mandated for all CAF and research personnel.

Animal cages are inspected for safety hazards prior to cleaning. Broken and bent wires on animal cages are repaired to ensure animal and human safety. Cracked plastic animal housing is discarded. Hypodermic equipment must be disposed of in proper containers. These devices are found in the CAF procedure rooms.

4. Miscellaneous

a. Animal-Associated Allergens

Many species of animals are known to cause allergies in humans. Reduction of exposure to animal allergens is recommended for all personnel working with animals. While a surgical mask will reduce exposure to hair and dander, only an N95 respirator (or equivalent) can adequately reduce animal allergen exposure. To reduce the risk of acquiring allergies, it is strongly recommended that an N95 respirator be worn during the handling of animals and their bedding to reduce allergen exposure. Safety glasses and protective clothing should routinely be used to prevent exposure to allergens and to prevent the transport of allergens outside of the animal room and facility. Rodent urine can produce severe allergic reactions and skin contact must be avoided; in the event of contact, wash off immediately with soap and water. All allergic reactions MUST be reported to the CAF supervisor and EOHS.

b. Glass and/or Sharps

Glass and/or sharps are to be disposed of in appropriate containers.

V. Reporting Safety Concerns

Individuals having concerns involving safety within Lifespan facilities are responsible for contacting the CRF Management, the Safety Office, the Research Administration Administrative Director, and/or the Research Protection Office, verbally or in writing. Contact information is provided to all researchers who work with animals during their initial orientation with the Central Research Facilities (CRF) Management. Telephone numbers for CRF management staff are posted within each animal facility. Contact information is also posted on the IACUC webpage. Complaints may be submitted anonymously to Corporate Compliance via the Employee Response Line at 888-678-5111.

Although written concerns are more convenient to handle, complainants may not be willing to submit them in this manner. In such cases, the individuals who receive concerns should document them fully to ensure that the issues are clear and to prevent misunderstandings.

Lifespan will take appropriate steps to protect the confidentiality of those who report concerns as well as anyone against whom allegations are directed, while allegations are under investigation.

Lifespan policy prohibits unlawful retaliation against employees as a consequence of good faith actions in the reporting or the participation in an investigation pertaining to allegations of wrongdoing.

VI. Veterinary Care

A. Role of Veterinary Care

Veterinary care at Lifespan is provided by board certified laboratory animal veterinarians through an agreement with Brown University. Lifespan has given assurance that the veterinarians have access to RIH management and have appropriate authority to ensure the provision of adequate veterinary care in the animal facilities.

The veterinarians are responsible for supervising a program of veterinary care which has been approved by the Institutional Animal Care and Use Committee and is in compliance with the Animal Welfare Act Regulations and the Public Health Services Policy on Humane Care and Use of Laboratory Animals. The program includes: (1) details on the facility, personnel, equipment, and services available for appropriate animal care; (2) acceptable methods to prevent, control, diagnose, treat health problems and injuries, and the availability of emergency weekend and holiday care; (3) guidance in the care and use of animals regarding handling, immobilization, anesthesia, tranquilization and euthanasia; (4) assurance that appropriate surgical areas for survival surgery are maintained and utilized, and that sterile technique is used; (5) assurance of adequate pre-procedural and post-procedural care including the appropriate use of anesthetics and analgesics; and (6) assurance that appropriate methods of euthanasia are utilized.

The veterinarians visit the CRF on a mutually agreed upon schedule and maintain frequent contact with the management of the Central Research Facilities. The veterinarians maintain an on-call schedule and are available in the case of emergencies after hours and on weekends or holidays. Their telephone numbers are posted in the animal facility.

The veterinarians also have frequent contact with CAF supervisors, the veterinary technicians, the Research O.R. Supervisor and the CAF technicians to discuss problems. The veterinary and animal care technicians are responsible for daily monitoring of the animals and recording changes in animal health, behavior, and wellbeing. Any health concerns or abnormal findings are reported to the veterinarian in an accurate and timely manner, via phone call, email, or in person.

The Director of the Central Research Facilities and the Chairperson of the IACUC are contacted about significant deficiencies or to propose changes in the animal care program or facilities. The veterinarians serve on the IACUC, the Animal Welfare Executive Committee, the Biohazards Laboratory Safety Committee and the Recombinant DNA Committee.

B. Veterinary Consultative Services

1. The veterinarians are available in person or via cell phone for consultation on a wide range of subjects including:

- Selection of appropriate animal species for *in vivo* studies.
- Information on animal models of human diseases.
- Anatomical and physiologic characteristics of individual animal species.
- Techniques of anesthesia, analgesia, chemical restraint., and euthanasia
- Design of appropriate post-operative care programs.
- Technique of collection and storage for blood, body fluids, and tissues.
- Effects of intercurrent animal disease on experimental results.
- Utilization of specialized surgical techniques.
- Experimental design.

2. Investigators are required to consult the Attending Veterinarian during the planning phase and prior to submission of the *Animal Care and Use Protocol* (ACUP). The consultation is indicated on the protocol forms. This consultation is used to advise or evaluate:

- the selection of experimental models

- consideration of alternatives to painful procedures
- directions and recommendations for the use of anesthetics and analgesics
- acceptable euthanasia methods, and the prohibition of the use of paralytics without anesthesia
- the qualifications and training of the investigator and staff to provide humane care for the animals, and to perform the procedures so that pain and distress will be minimized
- current laws and regulations concerning animal care and use

3. The veterinarians can provide health certificates for animal shipments from the facility.

4. The veterinarians, using external diagnostic facilities, when needed, evaluate clinical problems in all housed species and the veterinary technician or the CAF staff administers treatments under his guidance.

C. Reporting of Sick or Injured Animals (Clinical Medicine)

All personnel utilizing animal subjects are expected to contact Veterinary Services or the supervisor's office if they believe an animal is sick, in discomfort, or otherwise requires aid. A veterinarian will respond and take appropriate action in consultation with the investigator. It is essential that clinical calls be initiated at the earliest sign of an abnormality. The veterinarian will keep investigators informed of the diagnosis, condition, etc., and the appropriate course(s) of action.

1. Objectives

a. RIH is committed to providing veterinary care for all research animals in our facilities which is consistent with the objectives of the Institutional Animal Care and Use Committee approved protocol and in compliance with all applicable federal, state and local regulations and institutional policies.

b. The daily care of each research animal requires careful observation to detect potential complications due to research procedures and for unrelated injuries or disease. This is a shared responsibility of the personnel from the research laboratory and the staff of the Central Animal Facility.

2. Procedures

a. **Weekdays** - Business hours (7:00 AM – 4:30 PM)

Problems requiring prompt assistance:

Immediately contact Veterinary Services. (These numbers are posted on each floor of each research facility.)

- Main Campus mobile phone: (401) 585-8261*
- Off-Sites (Coro West and Claverick) mobile phone: (401) 255-4183*

*If unavailable, please contact the Attending Veterinarian (401) 444-6842 or cell (508) 250-2401 or call the CRF Main Office: (401) 444-5788.

Veterinary Services will contact the Veterinarian directly when on premises or by phone. They will assure that the animal is receiving appropriate attention and that appropriate documentation is maintained. This will include instructions from the

Veterinarian regarding immediate care instruction, diagnostic work up and treatment plan. For non-life threatening situations, every effort will be made to obtain approval from the PI or other laboratory personnel prior to initiation of treatment.

Veterinary Services will contact the CAF Supervisor regarding significant issues that might require further assistance or notification of CAF staff.

For problems requiring follow up assistance by Veterinary Services, the findings must be documented as completely and accurately as possible:

- For rodents, use one of the "Health Check" cards which are available in each of the rodent rooms and then post it on the animal's cage.
- For non-rodents, provide the required information for identification of the animal and the clinical problem on the animal's individual record.

b. **Off-Hours** (Weekdays and Weekends)

For animal health problems requiring prompt assistance, immediately contact the on-call veterinarian. In the event that the veterinarian cannot be reached, call the other number listed below.

- Main Campus: Vet Services Mobile: (401) 585-8261*
- Off-Sites (Coro West and Claverick) Mobile: (401) 255-4183*

Veterinary Services will then contact the Veterinarian on-call, as deemed necessary.

For problems requiring follow up assistance by Veterinary Services, follow procedures for section 2.A.4) above

c. **Weekend Daytime Hours** (7:00 AM – 3:30 PM)

For animal health problems requiring prompt assistance, immediately contact the on-call Veterinarian. In the event that the Veterinarian cannot be reached, call the numbers listed below for the CAF Technician on duty.

- Main Campus (401) 444-8146
- Coro West (401) 793-8761
- Claverick (401) 444-6978

If no answer, call the CAF Supervisor at (401) 255-4183 or (401) 585-8261.

The CAF Technician will contact the Veterinary Services staff member on-call as deemed necessary in consultation with the laboratory.

D. Utilization of Anesthetics and Analgesics

(See Appendix 3 Selection and Use of Anesthetics and Analgesics.)

Balanced anesthesia/analgesia will be employed to minimize surgical pain. A veterinarian must be contacted for assistance in designing appropriate anesthetic and analgesic regimens, which will be examined as part of the protocol review process conducted by the IACUC. Some agents have been shown to have undesirable physiologic effects which preclude their use in particular research situations. Investigators are urged to familiarize themselves with the agents used in their studies.

The following criteria should be considered in selecting agents for research studies.

- Species of animal(s)
- Is procedure acute or survival?
- Duration of anesthesia required
- Ease of administration
- Anesthetic effects
- Safety concerns
- Reversibility
- Recovery characteristics of the agents

General Principles:

- Large animals should be fasted the day before anesthesia (12 hours).
- Animals should be intubated to provide airway control during procedures.
- The animal's vital signs (HR, RR, temperature) must be monitored at least every 15 minutes during anesthesia.
- Emergency drugs should be available
- Animals must be monitored after anesthesia until they are fully awake.
- Animals must be able to maintain sternal recumbency and must have a rectal temperature of 99^{o} F or higher prior to return to their cage.

1. Pre-Anesthetics

Tranquilizers or sedatives are commonly used as pre-anesthetics for general anesthesia. Animals premedicated with sedatives and tranquilizers are more manageable and require lower dosages of general anesthetics.

2. General Anesthesia

a. Inhalational anesthetics should be administered using a precision vaporizer. Anesthesia machines regulate the flow of oxygen and the concentration of the anesthetic gas.

   Isoflurane delivered by mask or endotracheal tube via a precision vaporizer is recommended for all species. Vaporizers are available for use in the Claverick and Coro procedure rooms. Contact CAF for information regarding vaporizer availability and training. For very brief procedures in rodents, (e.g., tail biopsies for genotyping), it may be acceptable to use isoflurane or other inhalant anesthetics, without a precision vaporizer, in a “bell-jar” while precluding direct contact of animal skin with inhalant anesthetics. In all cases the anesthetic vapors must be adequately vented in a fume hood to prevent inadvertent exposure of personnel.
   Appropriate scavenging systems are required for personnel safety when using inhalational anesthetics. Additional information is found in the **SAFETY RULES** section of this manual.

b. Injectable anesthetics may be appropriate for some procedures. There is however, a great deal of variation in depth and duration of anesthesia between individual animals.

3. Local Anesthesia

The use of local anesthetics as an adjunct to other anesthetic protocols is encouraged. A local anesthetic is not required if the pain of giving the injection is as great and of the same duration as that produced by the procedure itself.

4. Analgesics

Analgesics are used in animal studies where pain may result from experimental manipulations. They should be used in animals for any procedures which require analgesics in humans.
Animals in pain may occasionally benefit from the effects of tranquilizers. Many tranquilizers provide muscle relaxation, sedation, and analgesia. *Appendix 3 Selection and Use of Anesthetics and Analgesics* provides dosages for the agents commonly used in animals.

E. Use of Controlled Substances in Animal Research

The Controlled Substances Act (CSA) was enacted into law by the Congress of the United States as Title II of the *Comprehensive Drug Abuse Prevention and Control Act of 1970*. The CSA is the federal U.S. drug policy under which the manufacture, importation, possession, use and distribution of certain substances is regulated. Controlled substances to be used for approved protocols must be obtained through the Rhode Island Hospital Pharmacy.

Investigators are responsible for the ordering, record keeping and security of any controlled substances required for their protocol. A log sheet showing the volume and use must be kept for each controlled substance. The RIH Pharmacy requires that the completed log sheet be returned to their department. Researchers may only order controlled substances from the RIH pharmacy with an active cost center.

Controlled substances must be kept under a double-locked storage system. In other words, you must open two locks in order to access the drugs. (e.g. double lock narcotic cabinet, a locked drawer in a locked room).

F. Pharmaceutical Grade Drugs

Consistent with USDA and PHS policy, investigators using Lifespan facilities are expected to use pharmaceutical-grade drugs or chemical compounds in all live animal research, whenever they are available (even for acute procedures).

Pharmaceutical grade substances are defined as those meeting pharmaceutical standards, being >99% pure, with no binders, filters, dyes or unknown substances. Lists of pharmaceutical-grade chemical compounds can be found in the human or veterinary physician's desk references (PDRs)

The use of non-pharmaceutical-grade drugs or chemical compounds is only permitted after specific IACUC review and approval. Approval for the use of non-pharmaceutical-grade drugs or chemical compounds will only be granted where:

- Acceptable pharmaceutical-grade substances are not available and/or,
- The use of the non-pharmaceutical-grade substance is scientifically necessary.

Note: Cost savings alone is not an adequate argument for the use of non-pharmaceutical-grade compounds in animals.

In reviewing requests for the use of non-pharmaceutical-grade substances, the investigator must describe preparation and at minimum, the procedures used to ensure sterility.

All non-pharmaceutical-grade substances must be sterile and maintained in sterile containers labeled with the name and concentration of the compound, as well as its expiration date. Heat-stable compounds may be sterilized by autoclaving, and those that are not heat stable can be sterilized by microfiltration. The investigator is responsible for determining the "shelf life" for the compound after being dissolved in solvent. If the "shelf life" is not obtainable, a fresh batch/aliquot of the solution must be mixed each day it is used.

For USDA and PHS/OLAW policies, go to:
http://www.aphis.usda.gov/animal_welfare/policy.php?policy=3 and
http://grants.nih.gov/grants/OLAW/references/lab_animal2003v32n9_wolff.htm

G. Standard Operational Procedures for Survival Surgery

1. Large Animal Survival Surgery

Aseptic surgical technique is used for all surgeries where the recovery of the animal is anticipated. In addition, all surgeries are to be performed in the areas approved by the IACUC as indicated in the ACUP.

Surgical procedures will be classified as either Minor or Major as evaluated by a veterinarian during the protocol preparation and approved by the IACUC. Typically, survival surgery will be classified as Major, where there is penetration of or exposes a body cavity, produces substantial impairment of physical or physiologic function, or involves extensive tissue dissection. Major surgical procedures will be conducted only in an operating room approved by the IACUC.

a. CRF Operating Room Scheduling

The operating rooms are scheduled on a first come first served basis. The Operating Room schedule is available for viewing on-line. Please note: Only investigators with approved large animal protocols will be granted access to the schedule.
It is recommended that you schedule your procedure(s) in advance to ensure availability of the room and any specialized equipment needed (ex. Fluoroscopy unit). Please contact the Operating Room Supervisor (444-6366) to ensure that any special needs can be accommodated and for instructions to access the OR schedule.

b. CRF Operating Room Charges

There are fees for the use of the operating room, technical assistance, and supplies. (See http://www.lifespan.org/research/administration/lifespan-core-research-services.html)

2. Rodent Surgery Overview

These Guidelines were developed to be consistent with those described in the *Guide for the Care and Use of Laboratory Animals* and any applicable requirements of the Animal Welfare Act regulations and Public Health Service *Policy for the Humane Care and Use of Laboratory Animals*.

- Adequately train all personnel to ensure that good surgical technique is followed.

- Conduct detailed pre-surgical planning to provide an opportunity for input from the surgeon, veterinarian, veterinary technicians, and the laboratory staff.
- Provide appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices.
- Conduct all survival surgical procedures in a designated surgery area in the laboratory, which is uncluttered and not being used at the same time for other laboratory procedures. Alternatively, Central Animal Facilities procedure rooms or surgical areas may be scheduled.
- Use aseptic procedures for all survival surgery, regardless of the interval of survival: if the animal recovers from anesthesia it is a survival surgery. This includes at a minimum wearing a cap, surgical mask and sterile gloves, using sterile instruments and practicing aseptic technique.
- Conduct a continuing and thorough assessment of the surgical outcomes to ensure that the appropriate procedures are followed and potential complications are detected and addressed. In the event of unanticipated morbidity or mortality, consultation with the Attending Veterinarian or designee is expected and appropriate corrective actions including amending the Animal Care and Use Protocol (ACUP) should be taken.

  The Principle Investigator and all personnel responsible for or performing rodent survival surgery must be trained in the following essential elements of good surgical technique. This training can be obtained through recommended on-line training in conjunction with hands on training by qualified personnel in the laboratory or by CRF Veterinary Services staff.

  - Asepsis
  - Gentle tissue handling including minimal dissection to avoid excessive tissue trauma
  - Appropriate maintenance and handling of surgical instruments
  - Effective hemostasis
  - Correct use of suture materials and patterns

In developing the protocol, the PI needs to:

- Develop the details for the survival surgical procedures conducted in rodents in consultation with the Attending Veterinarian or his/her designee.
- Provide a detailed description for each of the following:
  - Perioperative care and support including pre-operative medications, hypothermic prevention, ophthalmic protection (ointment)
  - Aseptic techniques including skin disinfection
  - Anesthetics and tranquilizers
  - Perioperative analgesics and anti-inflammatory agents
  - Nursing care and/or other treatments

- Provide a brief description of the area where the surgery will be conducted.
- Provide a description of the qualifications and training of personnel who perform perioperative care and survival surgical procedures in rodents.

See *Appendix 4 Guidelines for Survival Rodent Surgery* for details relating to disinfectants, sterilization methods, and recommended anesthesia/analgesia.

3. Post-Operative Care

It is the responsibility of the investigator, in consultation with the veterinarian and CRF personnel to provide appropriate post-operative care.

The veterinarian is available for consultation in designing protocol specific post-operative care programs. The following essential components should be routinely incorporated into post-operative management of rabbits and larger mammals

- The animal should be kept warm by the use of heating pads, chambers or lamps, and body temperature should be taken and recorded until it is normal (for most species this means a rectal temperature of 99^{o} F or higher).
- Animals should be rotated from side to side every 15 minutes until they are able to maintain sternal recumbency, and should not be left unattended until they have recovered consciousness and have complete control of their airway.
- Hydration should be assessed on a daily basis for at least three days after surgery. Any needed parenteral replacement fluids should be administered at a dosage of 40-60 ml/kg of body weight/day for animals which are not drinking post-operatively. Fluids should be given parenterally in animals which have had gastrointestinal procedures or which have depressed swallowing reflexes.
- Adequate nutrition is necessary in the healing animal. Caloric replacement should be instituted for animals that have not resumed eating by the second post-operative day. Caloric replacement may require supplemental feedings using specialized dietary formulations and feeding methods, or may necessitate intravenous hyperalimentation.
- Daily observations of the animals for alertness, activity, eating, drinking, and stool will be made for a minimum of three days post-operative or as otherwise stated in the protocol.
- The incision must be examined daily for evidence of wound dehiscence or infection. Sutures or wound clips should be removed 10-14 days post-operatively.

CRF Veterinary Services staff will work in concert with the laboratory personnel, Central Animal Facilities (CAF) personnel and the veterinarian to help ensure that animals receive high-quality post-operative care.

The CRF post-op form (*Appendix 5 Post Op Treatment Form*) must be used to record the progress of the animal post-operatively independent of any information that may be recorded in the investigator's laboratory notebook. Alternately, a PI may provide a different form if it captures the necessary information. All treatments should be entered as they are administered. The post-operative care form and/or information contained within lab notebooks are held by the investigator and made available to the CRF personnel, veterinarian and / or regulatory inspectors on request.

Although rodents do not generally require such intensive care, investigators should monitor their recovery from anesthesia, evaluate incisions and ensure that they continue to eat and drink post-surgically. Fluid and nutritional supplementation should be

instituted if necessary. For rodents, post-operative records should be kept for 3-7 days, depending on the type of surgery.

Emergency contact information for the persons responsible for post-operative care must be provided to the CRF office. This allows CRF staff to consult with research personnel in order to provide appropriate support or veterinary care to post-operative animals when problems arise, especially after hours, or during weekends and holidays.

H. Differentiating between Major and Minor Survival Surgery-Veterinary Perspective

1. Overall Concepts

The Eighth Edition of the NRC *Guide for the Care and Use of Laboratory Animals* offers much guidance on the major/minor surgical categorization issue (regarding research procedures, as opposed to veterinary clinical procedures) in the first two paragraphs under the heading Surgical Procedures on page 117. It states:

Surgical procedures are categorized as major or minor and, in the laboratory setting, can be further divided into survival and non-survival. As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993). Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time. When attempting to categorize a particular surgical procedure, the following should be considered: the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status and age of the animal.

Laparoscopic procedures and some procedures associated with neuroscience research (e.g., craniotomy, neurectomy) may be classified as major or minor surgery depending on their impact on the animal (Devitt et al. 2005; Hancock et al. 2005; NRC 2003; Perret-Gentil et al. 1999, 2000). For example, laparoscopic techniques with minimal associated trauma and sequelae (e.g., avian sexing and oocyte collection) could be considered minor, whereas others (e.g., hepatic lobectomy and cholecystectomy) should be considered major. Although minor laparoscopic procedures are often performed on an "outpatient" basis, appropriate aseptic technique, instruments, anesthesia, and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor should be evaluated on a case-by-case basis by the veterinarian and IACUC.

Note: The USDA has emphasized that any survival surgical procedure that goes beyond being considered as minor, must be categorized as major.

2. Practical Minor/Major Survival Surgery Differentiation at Lifespan

The veterinary recommendations to the Lifespan IACUC in differentiating minor and major survival surgery in the relevant species are as follows:

Rodents Minor survival surgical procedures in rodents should be limited to: tail biopsy and digit amputation commonly used for genotyping and identification; minimally invasive vascular cutdowns, subsequent artery/vein catheterizations and associated intravascular manipulations, and related incision closures; or to subcutaneous minimally traumatic tissue dissection and implantation of devices up to the size of osmotic pumps, and related incision closures. [Similar survival preparations that involve multiple manipulations and incisions, or significant tissue dissection, may (on a case-by-case basis) be considered by the veterinarian and the IACUC to be major surgery.] All open procedures invading a body cavity (i.e., thorax or abdomen), all procedures involving penetration of the cranium, and all procedures with more extensive/aggressive subcutaneous tissue dissection or which purposely injure or sever ligaments, tendons or muscle tissue, should be considered major survival surgical procedures.

Rabbits and Swine Minor survival surgical procedures in rabbits and swine are generally limited to skin biopsies, or to minimally invasive vascular cutdowns, subsequent artery/vein catheterizations and associated intravascular manipulations, and related incision closures. [Similar survival preparations that involve multiple incisions and/or manipulations may be considered by the veterinarian and the IACUC to be major surgery.] Depending upon the age, size and/or resiliency of the particular animals used in a study, the veterinarian may (on a case-by-case basis) consider some subcutaneous procedures with minimal tissue dissection and implantation of a compact/low mass (in relation to the size and body weight of the animal) foreign body as minor survival surgery. All open procedures invading a body cavity (i.e., thorax or abdomen), all procedures involving penetration of the cranium, and all procedures with more extensive/aggressive subcutaneous tissue dissection or which purposely injure or sever ligaments, tendons or muscle tissue, should be considered major survival surgical procedures.

3. Decision Making

Classifying survival surgical procedures as major or minor is a joint process involving the veterinarian and the IACUC, taken on a case-by-case basis (see page 30 of the Eighth Edition of the NRC *Guide*). Discussion and a sharing of viewpoints about a given preparation will, of course, take place during the ACUP review and approval process. The guideline here must be that in the event of a disagreement between the veterinarian and the IACUC, the most conservative categorization of what is to be done shall take precedence.

4. Suitable Sites for Non-rodent Mammalian Surgical Procedures

All survival surgery in rodents and in non-rodent mammals must be done aseptically. While rodent survival surgeries can be done in a designated space (generally a procedure room or a constant portion of a laboratory which is dedicated to surgery and related activities when used for this purpose), major survival surgery in non-rodent mammals certainly requires dedicated facilities. [The dedicated large animal surgical facilities at Lifespan are contained in the Research Operating Room Suite, 4th floor, Aldrich House.] Regarding functional areas in survival surgical facilities for non-rodent mammals, the Eighth Edition of the NRC *Guide* states on page 144: *For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery. The areas that support*

those functions should be designed to minimize traffic flow and separate the related non-surgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers (AORN 1993) but may also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities. The IACUC can consider requests by Principal Investigators to perform minor survival surgery in non-rodent mammals in appropriate designated space in procedure rooms or laboratory areas, with scientific justification, on a case-by-case basis.

5. Multiple Survival Surgical Procedures

The Eighth Edition of the NRC *Guide* states (in part) on page 30: *Regardless of classification, multiple surgery procedures on a single animal should be evaluated to determine their impact on the animal's well-being. Multiple major surgical procedures on a single animal are acceptable only if they are (1) included in and essential components of a single research project or protocol, (2) scientifically justified by the investigator, or (3) necessary for clinical reasons.* **As with major and minor surgical procedures, evaluation of requests for multiple survival surgical procedures is done jointly by the veterinarian and the IACUC on a case-by-case basis.**

I. Conditions for Multiple Major Survival Surgeries

If multiple major survival surgeries are being planned they should be related to a particular experimental endpoint and meet the following criteria to comply with PHS Policy and the Animal Welfare Act Regulations.

1. Any investigator requesting multiple survival surgeries must plan the project with the Attending Veterinarian or designee before submitting the protocol for IACUC consideration. This gives the Attending Veterinarian an opportunity to provide early guidance on how best to minimize pain, distress and/or discomfort to the animals.
2. The protocol submitted to the IACUC must include a description of the surgical procedures, the time frame for their performance and scientific rationale for doing multiple surgeries. Cost is not an accepted consideration in the IACUC protocol evaluation process.
3. In order to be considered for IACUC approval, the surgical procedures must be directed at securing a single valid objective.
4. If possible, the multiple procedures should be designed to cause less animal disability and/or morbidity than would a single complex procedure.
5. The proposed interval between procedures should be long enough to ensure an adequate recovery of the animal.
6. Patient monitoring capabilities for any multiple survival surgeries must be available and adequate.

Conservation of a scarce animal resource may justify the conduct of multiple major survival surgeries on a single animal, and will be reviewed critically by the IACUC. As part of the approval process, the Institutional Official must submit a request to the USDA/APHIS and receive approval.

J. Expired Medical Materials Policy

Most medical materials (e.g., drugs, fluids, disinfectant solutions, catheters, sutures, etc.) are imprinted with an expiration date. Beyond this date, the manufacturer does not guarantee the sterility, safety, or stability of the item. The use of expired materials without justification constitutes inadequate veterinary care under the Federal Animal Welfare Act.

For additional information, see USDA Animal Welfare Act Policy Statement (#3): Veterinary Care: Expired Medical Materials and Pharmaceutical-Grade Compounds in Research (http://www.aphis.usda.gov/animal_welfare/policy.php?policy=3)

1. Labeling and Storage of Expired Medical Products

Any medical or experimental material MUST be labeled as EXPIRED when it reaches the manufacturer's expiration date. We recommend the word EXPIRED be written across multiple sides of the container in RED or BLACK marker to assure staff will not inadvertently use expired product.

All expired products should be stored in an area separate from in-dated products. A separate cabinet is preferred, but if the same cabinet is used a separate box or other container to specifically hold the expired materials is recommended.

2. Use of Expired Medical Products in Survival Animals

The use of expired drugs or materials is prohibited in survival animals. All drugs and medical materials administered to live vertebrate animals must be used within their expiration date. This includes all fluids (such as saline & heparin) and all materials (such as sutures, catheters, etc.).

3. Use of Expired Medical Products in Non-Survival Animals

The use of expired drugs or materials is permitted in acute terminal procedures (i.e. where an animal is put under anesthesia, the research is carried out, and the animal is euthanized without ever waking up), if the use of the materials does not adversely affect the animal's well-being or compromise the scientific validity of the study.

The use of expired medical products in non-survival animals is subject to the following stipulations:

- Drugs administered to relieve pain or distress, including anesthetics and analgesics and emergency drugs (e.g., isoflurane, ketamine, dexdomitor, buprenorphine, fentanyl, etc.) **must** be within their expiration date. *The use of expired anesthetics, analgesics or emergency drugs is expressly prohibited*.
- Drugs used for euthanasia (i.e. barbiturates) **must** be within their expiration date. *The use of expired euthanasia drugs is expressly prohibited*.
- Expired drugs or fluids, beyond those outlined in 1) and 2) above (such as heparin or saline), may be used in non-survival animals without IACUC review. Consultation with the veterinarian is recommended, however, if there is any question as to whether an expired drugs or fluid may yield an adverse effect.

- Expired medical devices or materials (such as sutures, wound clips, catheters, etc.) may be used in non-survival animals without IACUC review. Consultation with the veterinarian is recommended, however, if there is any question as to whether the expired medical devices or materials may yield adverse effects.

The IACUC and/or attending veterinarian are responsible for ensuring that proposed animal activities avoid or minimize discomfort, distress, and pain to research animals. These responsibilities cannot be met unless the IACUC and veterinarian maintain control over the use of expired medical materials. Any discovery of expired medical materials being used inappropriately used **must** be brought to the attention of the IACUC or attending veterinarian.

K. IACUC Policy for the Humane Euthanasia of Laboratory Animals

1. General Background

a. Definition

The NIH *Guide for the Care and Use of Laboratory Animals* defines euthanasia as "the procedure of killing animals rapidly and painlessly". Techniques used for euthanasia must be chosen to assure that a rapid loss of consciousness will occur followed shortly by death without pain or significant distress being perceived by the animal.

b. Humane Considerations

There is a wide variety of animal species used in biomedical research, and specific methods used for each species must be considered based on their anatomy and physiology. However, the general principles for humane euthanasia in all species have been summarized by the International Council for Laboratory Animal Science (2006):

c. Principles for Animal Euthanasia

1. Whenever an animal's life is to be taken, it should be treated with the utmost respect.
2. Euthanasia should place emphasis on making the animal's death painless and distress-free. The method likely to cause the least pain and distress to the animals should be used whenever possible.
3. Euthanasia techniques should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and ultimate loss of brain function.
4. Techniques should require minimum restraint of the animal and should minimize distress and anxiety experienced by the animal, before loss of consciousness.
5. Techniques used should be appropriate for the species, age, and health of the animal.
6. Death must be verified following euthanasia and before disposal of the animal.
7. Personnel responsible for carrying out the euthanasia techniques should be trained:
   - to carry out euthanasia in the most effective and humane manner;
   - to recognized signs of pain, fear, and distress in relevant species;
   - to recognize and confirm death in relevant species.
8. Human psychological responses to euthanasia should be taken into account when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.

9. Ethics committees should be responsible for approval of the method of euthanasia (in line with any relevant legislation). This should include euthanasia as part of the experimental protocol, as well as euthanasia for animals experiencing unanticipated pain and distress.
10. A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little species-specific euthanasia research has been done. Gentle, careful handling of subject animals is of the utmost importance during the procedure in order to minimize distress to the animal. Measures should be taken to ensure that euthanasia is performed in a way that minimizes reactions among other animals that may be present. Euthanasia should be performed quickly and efficiently in a procedural area that is separate from rooms in which animals are housed. [Note: This is not always possible in a biohazard containment rodent room; in that case, euthanasia should take place in the room's Class II Biological Safety Cabinet.] When considering the impact of euthanasia on animal well-being, it is important to note that an unconscious animal does not perceive pain. Appropriately conducted procedures that render the cerebral cortex non-functional eliminate the perception of pain. Once this initial unconscious state is reached, reflex motor activity may still be observed, but pain is not perceived. This concept can be utilized in two-step approaches that combine an initial anesthetic event (e.g., general anesthesia via isoflurane or tricaine) with a secondary physical method (e.g., decapitation or exsanguination).

2. Best Practice Information

The primary source document for appropriate euthanasia practices is the *American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals:* 2013 edition. However, the committee writing that report recognized that it cannot be considered an all-encompassing document, and the language allows the use of professional judgment based on other current literature sources. The following reference list includes some of the most useful and readily available sources to be used when euthanasia methods are being considered.

a. Guidance

- *AVMA Guidelines for the Euthanasia of Animals (2013)*

American Veterinary Medical Association
http://www.avma.org/issues/animal_welfare/euthanasia.pdf

- *Guide for the Care and Use of Laboratory Animals (2011)*

Institute for Laboratory Animal Research
http://www.nap.edu/openbook.php?record_id=5140

b. Species-Specific Information

- *Report of the ACLAM Task Force on Rodent Euthanasia (2005)*
  American College of Laboratory Animal Medicine
  http://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf

3. IACUC Requirements

a. Protocol Requirements

Euthanasia is generally performed at the end of a project or, in some cases, at a point where animals would otherwise experience severe or chronic pain or distress that cannot be relieved. Because euthanasia may be needed as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments, protocols should include criteria for monitoring and initiating an early endpoint. This type of pre-planning for potential adverse outcomes will enable a prompt decision to be made by the research staff in conjunction with the veterinarian to ensure that the studies are humane and the objective of the protocol is achieved. Even when the planned experiment does not include euthanasia, there may be a need to humanely euthanize animals for unanticipated reasons. For this reason, at least one method must be documented for each species used in a protocol. Euthanasia techniques must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) during review and approval of the submitted protocol application form. Any subsequent change in euthanasia techniques must also be reviewed and pre-approved by the IACUC. The Office for Laboratory Animal Welfare (OLAW) characterizes the method of euthanasia as a significant component of the animal use protocol. Use of a euthanasia technique that is not described in the approved protocol may be considered significant noncompliance, which can result in protocol suspension and mandatory reporting to the federal funding agencies that support the Principal Investigator.

b. Training and Personnel Requirements

Euthanasia must be carried out by personnel properly trained in the procedure being used. This is especially important when physical methods such as decapitation or cervical dislocation are used as the primary methods, since these techniques require a certain amount of expertise to assure a humane outcome. It is the PI's responsibility to ensure that all persons performing euthanasia are properly trained and supervised. All individuals performing euthanasia as part of a research project must be listed on the approved protocol. The Veterinary Services staff of the Central Research Facilities (CRF) is available to demonstrate and/or discuss euthanasia techniques. Training forms must reflect species-specific euthanasia training. CRF personnel may provide euthanasia service for a nominal charge.

The CAF has refrigerators/freezers for disposal of small animals. The RIH Central Transport Department assists in the transportation of large animals to be disposed of by RIH. Radioactive animal carcasses should be disposed of in accordance with the guidelines in the ***RIH Radiation Safety Guide*.**

c. Verification of Death

Proper euthanasia technique will include a physical examination or close observation to assure that the animal is dead prior to disposal. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanized. Whenever possible, the best method is to confirm the absence of a heartbeat, which is a reliable indicator of death in most species. Monitoring respiration by observing chest movement is less valuable, because a heartbeat may continue after visible respiration has ceased. If respiratory movement is the only criteria, observation should continue for a prolonged period after euthanasia (e.g., 10-15 minutes for mammals).

Verification of death in animals can present special challenges. Unless total exsanguination or radical postmortem tissue harvesting (such as in the complete removal of the brain, heart, or lungs, or complete removal of vital internal organs with major blood supply such as the liver or both kidneys) will be certain to cause death, a secondary physical method such as decapitation, cervical dislocation or thoracotomy must be used to ensure death.

d. Equipment Used for Physical Methods

Physical methods may include the use of instruments that are blunt (e.g., cervical dislocation), or sharp (e.g., decapitation). Physical methods are approved with conditions per the AVMA Guidelines with the conditions being that the operator demonstrates competence in the technique and that the instruments used are appropriate. The Principal Investigator (PI) must ensure that all personnel that perform euthanasia are appropriately trained and have demonstrated competence in the technique. The PI must also ensure that the choice of instrument is appropriate for the size and the anatomical conformation of the animal involved, with input from the Attending Veterinarian as needed. In many cases the use of specialized equipment such as a custom guillotine or enterotomy scissors will perform better than conventional scissors, knives or scalpels. Each lab must provide for the proper periodic evaluation and sharpening or replacement of equipment to assure proper function and document the regular maintenance of the equipment.

e. Study Considerations and Alternatives

It must be recognized that it is extremely important for experiments be planned and performed in a way that ensures the validity of the data produced. If the euthanasia method used interferes with the ultimate goals of the research study and makes the data unusable, then the lives of the animals may have been wasted. Careful consideration of the possible adverse effects of the various options available must occur. There may occasionally be special circumstances or situations in which options that are not listed in this document might be considered acceptable. These exceptions must be carefully considered by the investigator and the IACUC to assure the best outcome for the animals as well as the study.

f. Disposal of Carcasses

Prior to placing the carcass in a cooler or freezer, put it into a bag and label it with the name of the PI, IACUC Committee number, initials and the date and method of euthanasia (both primary and secondary if applicable). This applies to all species.

4. Recommended Agents and Methods of Euthanasia Listed By Species

The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol. Generally, inhalant or non-inhalant chemical agents (such as barbiturates, inhalant anesthesia, or CO2) are preferable to physical methods (such as cervical dislocation or decapitation). However, scientific considerations might preclude the use of chemical agents for some experimental studies. All methods of euthanasia must be reviewed and approved by the IACUC.

The table at the end of this section provides information about Lifespan IACUC approved methods of euthanasia for various animal species and ages.

a. Rats, Mice, and other Small Mammals

- Inhalant anesthesia (isoflurane) except in animals under two weeks of age. Note: Must be followed by a secondary physical method, such as cervical dislocation or decapitation.
- Carbon dioxide (CO2) except in animals under two weeks of age. Note: Must be followed by a secondary physical method, such as cervical dislocation or decapitation.
- Barbiturates (given intraperitoneally or intravascularly) at any age.
- Exsanguination (under general anesthesia)
- Physical methods such as decapitation (especially in mice and rats less than one week of age) or cervical dislocation performed by a trained individual with demonstrated competence in the technique being used.

b. Rabbits

- Barbiturates (given intravascularly)
- Exsanguination (under general anesthesia)

c. Swine

- Barbiturates (given intravascularly)
- Exsanguination (under general anesthesia)

d. Dogs and Cats

- Barbiturates (given intravascularly)
- Exsanguination (under general anesthesia)

5. Technical Comments on Agents and Methods

a. Inhalant Anesthesia

Because most inhalant anesthetics act as topical irritants in their liquid state, animals should be exposed to the vapors of the anesthetic only. Chambers must be designed to assure the animals don't come into contact with the wicking material that may be saturated with the liquid phase of the anesthetic. Sufficient air or oxygen must be provided during the induction period to avoid hypoxia prior to unconsciousness. All agents are given "to effect" until respiratory and cardiac arrest occurs. ***In order to assure mortality after inhalant anesthesia in those circumstances where death is not always a certainty (see Verification of Death in Section 3.c., above), a secondary physical method must be employed prior to disposal.*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity). Isoflurane is the only inhalant anesthetic approved for animal euthanasia at Lifespan.

b. Non-Anesthetic Gas

(NOTE: The Lifespan Policy on Carbon Dioxide Euthanasia must be followed, and the use of special equipment is required. See Required Use of Flow Regulators for CO_2 Euthanasia of Rodents below)

Carbon dioxide has long been the preferred technique for euthanizing rodents over two weeks of age and other small laboratory animals. Use of a sealed chamber filled

by a compressed gas cylinder is required. CO_2 generated by other methods, e.g., dry ice, is unacceptable. Chambers must not be overcrowded to avoid distress during the procedure. Because CO_2 can act as a reversible anesthetic, it is imperative that the animals be kept in the chamber for at least one minute following the cessation of respiration. ***In order to ensure mortality after CO_2 exposure, a secondary physical method must be employed prior to disposal (see Verification of Death in Section 3.c. above).*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity).

Carbon Dioxide Euthanasia Procedures for Mice and Rats
(Lifespan IACUC Recommended)
Note: these procedures are <u>not</u> suitable for neonatal (< 2 weeks of age) animals.

1. Use compressed CO_2 from a cylinder affixed with a regulator. (The use of dry ice or other sources of CO_2 is prohibited.)
2. Avoid overcrowding or mixing of unfamiliar or incompatible animals.
3. Avoid pre-filling the euthanasia chamber with CO_2 prior to placing animal(s) in the chamber. Residual CO_2 (e.g., when the chamber contains CO_2 from recent use) is not acceptable so care must be taken to empty and clean the chamber between uses.
4. Use a low flow rate of CO_2 to gradually increase the CO_2 concentration, a 10-30% volume displacement rate is required.
5. Monitor for adverse reactions. Contact the Veterinary Services staff if the procedure appears to produce excessive agitation or other complications.
6. Leave the animal(s) in the high CO_2 environment for a minimum of 5 minutes following loss of breathing and apparent death.
7. Verify euthanasia (death) and perform a secondary method prior to disposal.

Required Use of Flow Regulators for CO_2 Euthanasia of Rodents

The American Veterinary Medical Association (*AVMA) Guidelines for the Euthanasia of Animals: 2013 Edition* **mandate that pressure-reducing regulators and flow meters (or equivalent equipment) be used during CO_2 euthanasia of rodents, to provide an environment of controlled, gradually increasing CO_2 concentration.**

The rationale for the use of controlled, gradually increasing CO_2 concentrations is that CO_2 euthanasia can cause distress via: (1) triggering pain due to the formation of carbonic acid on respiratory and ocular membranes, (2) the 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. **Without flow regulators it is impossible to adequately control CO_2 chamber filling to the level required by the**

new Guidelines. The optimal flow rate for CO_2 euthanasia systems is one that yields a displacement rate of 10% to 30% of the chamber or cage volume per minute.

Accordingly, **regulator/flow meter systems are required for all CO_2 euthanasia stations on the Rhode Island Hospital Campus and in the Lifespan-affiliated research facilities (CORO, Claverick, and Kilguss)**. New regulators and flow meters have been installed in the public CO_2 euthanasia stations within the CRF facilities. **Investigators who desire the convenience of performing CO_2 euthanasia in their laboratories must purchase and install appropriate CO_2 regulator and flow systems.**

c. Pharmacological Agents

Use of pharmacological agents requires adequate appropriate physical restraint and mastery of appropriate injection techniques. Barbiturates are acceptable for all species, but are most commonly used for mammalian species and birds. These drugs should be administered intravenously (IV) whenever possible, but intraperitoneal (IP) administration is acceptable for rodents. Sodium pentobarbital is the most common barbiturate agent for euthanasia, used either alone or in commercially available euthanasia mixtures. The dosage is usually at least twice that required for anesthesia. A dosage of 120 mg/kg is sufficient for most species, but more should be given if death does not ensue. Commercial euthanasia formulations should be used following label directions (e.g., 1 ml/10 lb for Beuthanasia-D). Sodium pentobarbital is a Class II controlled substance that is tightly regulated. Investigators using this agent are required to store the drug in a locked location and maintain detailed use records. An overdose with non-barbiturate injectable anesthetic (e.g., ketamine/dexmedetomidine or ketamine/xylazine) is not acceptable as a sole method, but such drugs can be used to sedate or anesthetize animals prior to the use of a physical method in a two-step procedure. ***In order to ensure death after the use of pharmacological agents a secondary physical method must be employed prior to disposal (see Verification of Death in section 3.c., above).*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity).

d. Physical Methods - (NOTE: Physical methods require that the user have experience and skill in the techniques to be used.)

- **Exsanguination** is acceptable for all species under general anesthesia. Rapid removal of blood can be accomplished by severing major vessels or (in smaller animals) by cardiac venipuncture.
- **Cervical dislocation** is acceptable for mice and rats weighing less than 200 gm, but proper technique is essential. Individuals performing this technique must receive prior training and have demonstrated competence in its use.
- **Decapitation** with proper equipment may be performed on mice and rats. Decapitation using sharp scissors or a blade is a preferred method for mice and rats less than one week of age. Individuals performing this technique must receive prior training and have demonstrated competence in its use. Many species

react adversely to the smell of blood, so animals should not be decapitated in the presence of other animals and the person performing decapitation should change gloves and/or wash hands between animals.

IACUC Recommended Euthanasia Methods			
Species	Age/Wt	Method/Route/Dose	Comments
Mice/Rats	≥ 14 days	CO_2/Inhalation/to effect	Follow Lifespan Procedures (above) Secondary physical method required
Mice/Rats	All	Barbiturate/IP/≥ 120 mg/kg	
Mice/Rats	All	Isoflurane/Inhalation/to effect	Secondary physical method required
Mice/Rats	≤ 7 days	Decapitation	Sharp blade or scissors; demonstrated competency
Mice/Rats	All	Decapitation	General anesthesia or justification with demonstrated competency
Mice/Rats	≤ 200 g	Cervical dislocation	Demonstrated competency
Mice/Rats Rabbits	All	Exsanguination	General anesthesia
Rabbits	All	Barbiturate/IV/≥ 120 mg/kg	Ear vein or other suitable vessel
Swine	All	Barbiturate/IV/≥ 120 mg/kg	Ear vein or other suitable vessel Typically sedated
Dogs/Cats	All	Barbiturate/IV/≥ 120 mg/kg	Cephalic vein or other suitable vessel

Unintended recovery of animals after apparent death (e.g., found alive in morgue) constitutes a SERIOUS NONCOMPLIANCE with the PHS Policy and serious deviation from the provisions of the *Guide for the Care and Use of Laboratory Animals*. Any incidents of unintended recovery must be reported to the IACUC and OLAW.

L. Animal Health Program

Lifespan maintains an animal health program of vaccinations, physical examinations and assessments. Refer to *Appendix 6 Animal Health Program* for a list of normally administered vaccinations for large animals.

M. Animal Health Surveillance

- Diseases in rodents are known to alter research results. Several bacterial and mycoplasmal diseases manifest themselves clinically after long incubation periods or only after experimental stress. Inapparent viral diseases have been shown to have immunomodulatory effects. Therefore, the veterinary staff recommends that

investigators utilizing rodents as animal subjects purchase them from vendors that maintain stocks and strains free from murine pathogens.

- Surveillance programs are instituted to monitor in-house colonies of these animals to ensure that their microbiological integrity has remained unchanged.
- The veterinary personnel, in conjunction with commercial laboratories, provide surveillance under the animal health program protocol. Periodic submission of sera for virus and mycoplasmal antibody testing and parasitological exams is recommended (*Appendix 6 Animal Health Program*).

N. Rodent Health Monitoring Program

1. Overview

The health status of the rodent colonies, which include all rodent holding rooms in Middle House, Coro and Claverick, are monitored on a quarterly basis for the early detection of viral and/or parasitic infections that could compromise animal health and/or the interpretation of research results. The program utilizes sentinel rodents which have been exposed to soiled bedding from the study animals housed in the same location. The sentinel mice then undergo quarterly testing for endoparasites (direct examination of gastrointestinal contents and fecal flotation and tape test), ectoparasites (fur pluck tape test) and viral pathogens (serology testing). These quarterly results are available to all Rhode Island Hospital investigators conducting animal research and are also available to external facilities wishing to import rodents from our facility. If a potential contamination is detected, researchers are promptly informed as described below.

2. Response to Positive Murine Pathogen Findings in Lifespan Facilities

CRF Management Team: CRF Director, Veterinarians, CRF Supervisors/Coordinators

CRF Administration: Vice President for Research, Administrative Director-Research Administration, IACUC Chairperson and Vice Chairperson, Director-Research Protection Office, IACUC Coordinator

GENERAL PRACTICE

- The Veterinary Services Supervisor will forward all laboratory results to the veterinarians the same day of receipt.
- Within 24 hours of receipt the veterinarians will review the reports and make a determination as to whether the findings warrant action.
- ***If the findings do not warrant action***, the veterinarians will inform the CRF Director and CRF Supervisors/Coordinators via email of the results and their interpretation.
- The veterinarian's summary of all non-actionable findings will be forwarded to the general research community via email within one week of receipt by the CRF Director.

UNANTICIPATED FINDINGS

1) ***If the findings do warrant action***, the veterinarians will immediately:
   - Inform the CRF Administration via email,
   - Inform the CRF Director via email or telephone call, and
   - Contact the Veterinary Services Supervisor to initiate Confirmatory Testing

2) Upon receipt of the veterinarian's notice of actionable unanticipated findings, the CRF Director will immediately:
   a. Instruct the Supervisors/Coordinators to increase the level of containment in the affected and any "at risk" rooms, including:
      - Cessation of movement of animals into or out of the affected room(s),
      - Posting of a notice on the door(s) to the affected rooms describing the results of the pathogen testing, the need for heightened containment measures, and any special sanitary precautions. The notice will include contact information (pager and cell phone numbers) for the veterinarians and the CRF Director
   b. Recruit the CRF Compliance Coordinator to open a Pathogen Outbreak file and begin documenting the outbreak and CRF response. The file will remain open and be updated regularly until the outbreak is eradicated.
      - A brief note will be written in the file each week day by the CRF Director or veterinarians to document progress, assess compliance with procedural issues and make recommendations for modifications.
   c. Notify all affected Principal Investigators via email
   d. Organize a meeting of the full CFR Management Team to review the unanticipated findings. The meeting will be held within 48 business hours of the veterinarian's notice to CRF. The purpose of the meeting will be to discuss the unanticipated findings, the timeframe and potential outcomes of the confirmatory testing, and potential modifications of the heightened containment measures. The goals of the meeting will be to:

      Define and Contain the Outbreak
      - Define the areas of "presumed" contamination and areas of "likely" contamination (based on the characteristics and transmissibility of the infectious agent).
      - Identify potential cross-over areas that may require additional disinfection procedures, and define disinfection procedures.
      - Define modifications to garbing/protective gowning practices required in the facility

      Develop a Plan for Continued Surveillance
      - Review the status of the Confirmatory Testing outlined in 1) above
      - Develop recommendations for additional confirmatory and surveillance testing throughout the facility for all three possible confirmatory test outcomes: negative, positive or equivocal

      Modify the Standard Animal Husbandry Plan
      - Document the number of affected Investigators, IACUC Protocols, and animals in the presumed and likely contaminated areas
      - Define standard husbandry procedures in the affected areas
      - Generate recommendations for the modification of the standard husbandry procedures to minimize the risk to other areas of the facility and Institution

      Generate a Tentative Plan for Animal Disposition
      - Develop recommendations for disposition of animals in the affected rooms (and facility, if necessary), which may include depopulation.

e. Coordinate individual or group meetings between any affected Principal Investigators, the veterinarians and CRF Director

(Note: It is expected that the CRF Director, Supervisors and Coordinators will contribute to the discussions outlined in 2d., above. However, the ultimate responsibility for the final Containment, Surveillance, Husbandry and Depopulation Plans rests with the veterinarians.)

3) By the time the results of the Confirmatory Testing are received (typically within 7 days) the veterinarians will have generated a brief report of the outbreak and a Tentative Management Plan, with contingency recommendations for negative, equivocal and positive findings (Table 1, below).

   The veterinarians will forward their tentative management plan and recommendations to the members of the CRF Administration and the CRF Management Team as soon as they are completed.

Table 1: Options for Tentative Management Plan

Confirmatory Testing Results	Potential Courses of Action
Negative Results (veterinarian Interpretation)	**1) <u>No Additional Surveillance</u>** • Return to normal operations **2) <u>Continued Surveillance</u>** • Recommend testing interval and duration of follow-up confirmatory testing • Recommend modifications to level of containment
Equivocal Result (veterinarian Interpretation)	**<u>Continued Surveillance</u>** • Recommend testing interval and duration of follow-up testing • Recommend modifications to level of containment
Positive Result (veterinarian Interpretation)	**1) <u>Continued Surveillance</u>** • Recommend testing interval and duration of follow-up testing • Recommend modifications to level of containment **2) <u>Colony Disposition</u>** • Recommend plan for colony disposition/depopulation

RESULTS OF CONFIRMATORY TESTING

1) Upon receipt of the results from the confirmatory testing the veterinarians will immediately:
   - Inform the CRF Administration via email,
   - Inform the CRF Director via email and telephone call
2) Upon receipt of the veterinarians' notice, the CRF Director will immediately:
   a. Notify all affected Principal Investigators
   b. Coordinate a "Town Hall" meeting for all interested researchers to convey the results of testing and the Management Plan.

- The meeting will be scheduled within 72 hours of receipt of the Confirmatory Testing results.
- Researcher invitations will be via email and will include a description of the outbreak, the results of the confirmatory testing, and the tentative plan for containment or eradication.
- Presenters at the town hall meeting will include, at minimum, the veterinarians, the CRF Director, the Administrative Director of Research Administration, and the IACUC Chairperson/Vice Chairperson.

3) ***If the confirmatory testing results are negative or equivocal***, the veterinarians will contact the CRF Director to begin execution of the appropriate sections of the Management Plan.
   - If additional surveillance is necessary, the plan will be modified using the process outlined in item 2c, as necessary.
4) ***If the confirmatory testing results are positive***, the veterinarians will contact the CRF Director to begin execution of the appropriate sections of the Management Plan.
   - If additional surveillance is necessary, the plan will be modified using the process outlined in item 2c, as necessary.
5) The CRF Director will send progress updates to all PI's weekly until the offending pathogen has been eradicated.

O. IACUC Policy for Tumor Implantation

Purpose

A. To provide guidelines for a tumor implantation and monitoring for mice or rats inoculated with neoplastic cells or toxic agents or animals that are genetically predisposed to develop tumors. This guideline is relevant to all investigators using models of neoplasia, including all subcutaneous, liquid, or non-palpable tumors; in addition, it applies to naturally occurring tumors. Humane interventions and endpoints should be determined and specified in the Animal Care and Use Protocol (ACUP) for all animals that will undergo tumor development as an expected part of the experimental protocol.

B. To describe the procedures for monitoring and documenting animals on protocols involving experimentally induced tumors.

C. To provide guidelines for evaluating the overall health of the animal and applying humane endpoint criteria.

Tumor Implantation Sites

Tumor implantation sites should be chosen to minimize adjacent tissue damage or disrupting normal physiology. **The IACUC recommends implanting tumors on the dorsum or flank of an animal**, as these areas will likely have the least amount of site-related morbidity. If other sites are to be used, describe and justify in the ACUP.

- Sites involving the face, limbs or perineum should be avoided as there is little to no space for tumor growth and expansion, and they may interfere with eating and drinking.
- Intramuscular implantation should be avoided to prevent inhibiting normal movement

- Tumor implantation on the abdominal surface of the body should also be avoided due to the risk of irritation to the tumor site in contact with the bedding and floor of the cage.

Tumor/Clinical Evaluation

Evaluation of visible or palpable tumors

Evaluating tumor burden based only on a percentage of body weight is generally not accurate while the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may be decreased (loss of lean body mass), resulting in a relatively stable body weight but an unhealthy animal.

Tumor burden should be determined by evaluating the following:

- Body Condition Score (BCS); see below. Alternatively, for liquid tumors body weights may be used.
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors
- Tumor ulceration

[The following guidance assumes that a normally sized adult rodent will be studied (a ~25 g mouse or a ≥250 g rat). The allowable sizes of tumors will be decreased if the tumors are injected into immature or genetically small mice.]

Tumor Size and Location

The concern of size for individual tumors is related to central necrosis, ulceration of skin overlying tumors, and abrasions. When on the dorsum or flank of adult rodent, tumors may be allowed to grow to the following volumes as long as the rodent remains otherwise healthy.

- Mice: 2000 mm^3 in size (which is roughly 10% baseline body weight),
- Rats: 5000 mm^3 in size

(For the basis of this policy, tumors may be measured using the following formula:
$TV = [(Width)^2 \times Length] / 2)$

Multiple Tumors

Multiple tumors that are individually smaller than the single tumor limit may not have the same negative sequelae as a single tumor. Multiple tumors may be allowed to grow up 150% (or 3000 mm^3) of the volume compared with the volume of a single tumor. Please note that the limitation on any single tumor (2000 mm^3 volume in mice) will still be valid.

Tumor Ulceration

Ulceration (overt open lesion or scabbed area) of a tumor typically requires euthanasia UNLESS justified in the protocol and in consultation with the veterinarian, and will require at least daily monitoring.

Non-palpable or liquid tumors

Evaluating liquid tumors (e.g. leukemia) and tumors in central areas of the rodent's body (e.g. bone, brain and lungs) can be challenging. Tumor size will likely not be useful due to inability to measure size or because of the sensitivity of areas to compressive lesions. For these models, the BCS AND/OR body weight along with clinical evaluation of the animals take priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints of those signs must be clearly described in the protocol. A scoring system (as mentioned above in this document) may be most helpful in this scenario. The evaluation of clinical signs in an animal with a tumor burden of this type should include consultation with a veterinarian.

Tumor Monitoring Procedures

A. Principal investigator or designated lab member

1. Identify each cage at the time of injection of tumor cells, cage cards must be identified with an identifying tag. Tumor monitoring must begin at this time per protocol specific frequency **(or at least once per week, whichever is more frequent).** After a visible or palpable tumor is evident, the animals must be monitored at least twice weekly. More frequent observations may be necessary as determined by the veterinarian, based on tumor growth rate, study parameters, and general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal will take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.
2. Provide each cage with a unique cage number on the identifying tag using a permanent marker. (This is intended to facilitate communication between the research laboratory and the animal care staff and veterinarians.)
3. A tumor monitoring sheet must be filled out for each protocol endpoint. The monitoring sheet must be filled out completely indicating:
   - protocol specific endpoints
   - monitoring frequency
   - contact information for the person who is directly working with the animals

   For each observation fill in date, observation code, cage identification numbers, and initials. For observations (U) ulcerated, (D) found dead and (E) euthanized, record number of animals with the observation code

B. Veterinary Services Staff

1. Inspect the tumor monitoring sheet at least once a week (same day each week).
2. Notify the laboratory, in writing, that "tumor monitoring sheet upkeep" is required if not adequately completed and needs to be completed in the next 24 hours.
3. Examine any animal of concern during the standard daily animal health checks and report at least the following:
   - any tumor reaches the size of a dime (18 mm)
   - any tumor which inhibits mobility
   - skin ulceration noticed at the tumor location
   - clinical signs including loss of body condition

4. Verify the tumor monitoring sheet for completeness and consistency with the protocol for the following:
    - laboratory contact
    - protocol number
    - cage identification number
    - tumor monitoring frequency
    - protocol endpoint
5. Contact the responsible laboratory member as needed.
6) Report any communication issues to the veterinarian.

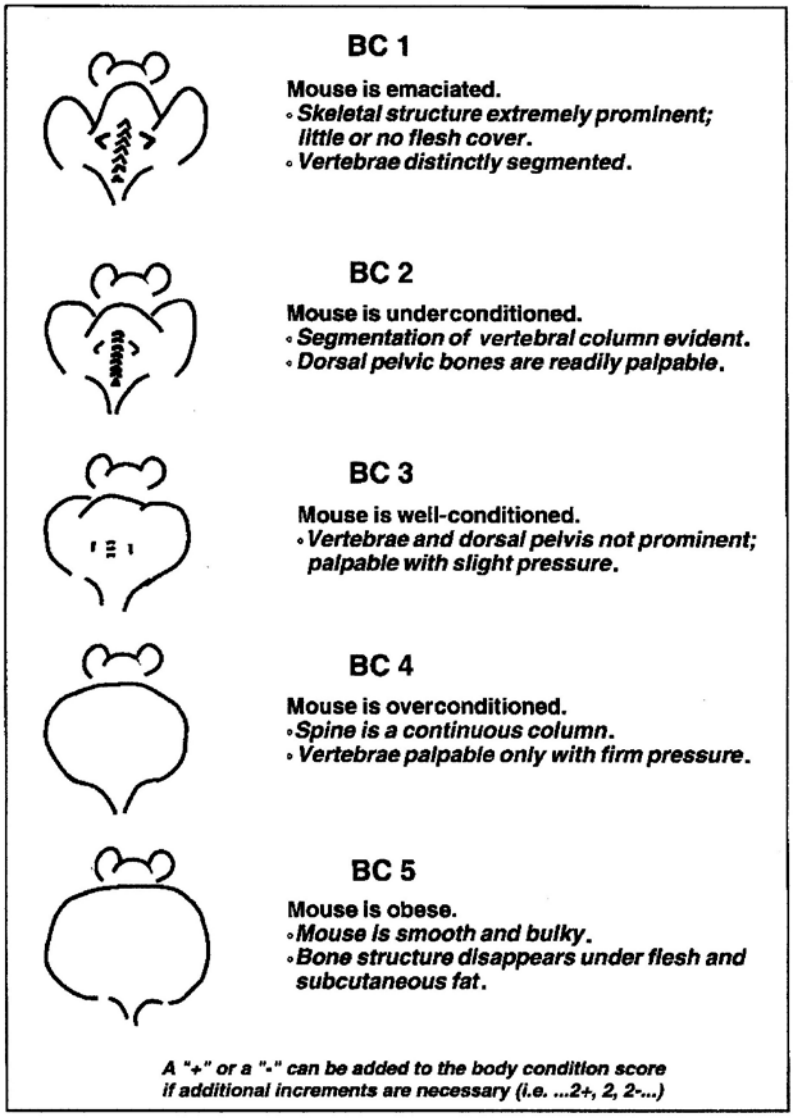
Animal Assessments

A. Body Condition Score (BCS)

The general physical condition of the animal is an important factor in effectively following the progression of tumors in rodents. Scoring systems from "1" (emaciated/wasted) to "5" (obese) are often used. BCS is a helpful adjunct to assessment of overall health of the animal. It is important to note that treatments designed to affect tumor growth (such as chemotherapeutics) which are often part of tumor load studies, can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumor load experiments.

Rodents must be euthanized if:

- The body condition score is 1/5
- The body condition score is 2/5 and the mouse has decreased activity/responsiveness
- The tumor affects the rodent's gait or normal posture, ability to eat, urinate, or defecate independent of the size of the tumor
- The veterinarian determines that the animal should be euthanized for humane concerns



i. General clinical signs should be assessed. Any evidence of lethargy or other change in behavior, change in ambulation, diarrhea, neurological signs (e.g. circling, head tilt) or increased respiratory effort need to be reported to the veterinary staff.

ii. The known biology and effects of any individual tumor model will be described in the ACUP, including expected clinical signs, anticipated moribundity/mortality, interventions for the relief of pain and suffering, and objective criteria for the assessment of humane endpoints.

iii. Any animal which is found to be at protocol endpoint or which meets the guidelines for endstage illness must be euthanized.

The professional judgment and decision of the Attending Veterinarian is final.

Reference: Wallace J. Humane endpoints and cancer research. ILAR J 2000; 41:87-93.

Utilization of transplantable tumors, cell lines and other biologics

Transplantable tumors, cell lines, and biologicals which have been passaged in animals may be contaminated with viable pathogens present in those animals. Murine viruses have inadvertently contaminated rodent colonies in this way and there is a potential for pathogen transfer in all species. All transplantable tumors, cell lines, and other biologicals with previous passage in animals must be tested for adventitious pathogens prior to use at Rhode Island Hospital. The CRF Director or veterinarians can provide additional information on testing options.

Biologicals posing special hazards to humans must also be approved by the Biohazards and Laboratory Safety Committee. Organizations that provide biological materials, e.g. ATCC, typically do not test for these agents. Biologicals typically require additional testing in order to detect possible infectious contaminants before passage occurs in animals at Rhode Island Hospital.

In addition to obtaining IACUC approval, Investigators must obtain approval from the Biohazards and Laboratory Safety Committee (BLSC) to utilize particular biologics in animals within the facility.

P. Policy on Use of Human Source Tissues and Cells in Immunodeficient Animals

Human source tissues and cell lines may carry human or zoonotic pathogenic or adventitious agents. When placed in immunodeficient animals, such as nude or SCID mice, these agents have the opportunity to replicate and may present a risk to scientific and animal care staff.

ATCC does not test all cell lines for human pathogens, in fact, some are known to be positive for human pathogens. The organization recommends that viral testing should be performed on their cell lines, especially when culturing cell lines in an animal facility or *in vivo* conditions. ATCC recommends: "Please keep in mind that all adventitious agents may not be detected through viral testing. For this reason we strongly recommend that all human and other primate cell lines be handled at the same biosafety level as a cell line known to carry HIV or hepatitis virus."

Immunodeficient mice and rats carrying human cells or tumors will be housed at Animal Biosafety Level 2 (ABSL-2).

See supporting references: CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and CDC Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories

Q. Prolonged Restraint

In general, restraint for all animals should be the least restrictive and for the shortest time necessary to complete research objectives. Prolonged restraint should be avoided unless it is essential for achieving research objectives. Examples of prolonged restraint include primate chairing, rodent restraint in inhalation chambers, and swine and dogs restrained in slings. Consider the following guidelines:

1. Restraint devices are not to be considered normal methods of housing.
2. Restraint devices should not be used simply as a convenience in handling or managing animals.

3. The period of restraint should be the minimum required to accomplish the research objectives.
4. Animals to be placed in restraint devices should be given training to adapt to the equipment and personnel.
5. Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.

Veterinary care should be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates temporary or permanent removal of the animal from restraint.

R. Environmental Enrichment Program for Laboratory Animals

1. Objectives

The objective of the Environmental Enrichment program is to provide the research animals housed in Rhode Island Hospital research facilities with living environments which allow for expression of noninjurious species-typical activities. This is required by the USDA Animal Welfare Act (AWA), the *Guide for the Care and Use of Laboratory Animals* (the *Guide*), and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The purpose is to enable animals to cope with the stresses of confinement and research procedures by allowing them some degree of freedom to manipulate their environment. Environmental enrichment may reduce stereotypical/ repetitive behaviors and promotes the health and well being of the animals.

2. Definitions

*Manipulanda – Any objects that can be manipulated by an animal or encourage it to engage in fine motor movements. Such as wooden blocks or prefabricated plastic chew toys.

3. Details of Procedures

a. General

- All animals will be provided environmental enrichment, which is considered beneficial for that species.
- When exemptions to this SOP are required due to study restrictions they must be justified by the Principal Investigator to the IACUC who will evaluate the request based on scientific grounds. The IACUC has sole authority to grant exemptions. The PI can request the exemption in the ACUP or by an amendment. An alternative enrichment will be proposed whenever possible.
- The Attending Veterinarian is charged by the IACUC for overseeing the Environmental Enrichment program as described in this SOP, and does have the authority to restrict environmental enrichment for medical reasons. Restrictions must be in writing and renewed monthly. Veterinary exemption will be noted in the animal's record.

- The Central Animal Facility management is charged with ensuring the implementation of all procedures. The CAF care staff will be responsible for carrying out this program.
- The CAF Supervisors will be responsible for periodically evaluating the condition of all environmental enrichment devices (manipulanda) and disposing of any items that are severely chewed, contain sharp edges or are otherwise broken or unsanitizable. Manipulanda will be changed, sanitized, or discarded at least every 2 weeks at the time of cage cleaning. Reusable manipulanda will be cleaned and disinfected in the cage washer.
- Toys/devices will be selected and maintained with respect to the safety of the animals. The animal care staff will notify the CAF Supervisor of any problems or potential problems with enrichment items.
- The environmental enrichment program will be re-evaluated periodically based on investigator and CRF staff feedback.
- An enrichment program will be developed for new species prior to the species being received in the CRF. The IACUC is responsible for notifying the Attending Veterinarian and CRF Director of plans for adding the new species to the program. The Attending Veterinarian and Director will decide on the best items and methods to use to provide enrichment and will amend this SOP.

b. <u>Enrichment Details</u>

At least one enrichment method is always used from one of the following enrichment groups (comprising Manipulanda, Nutritional, and Socialization/Environmental) listed below.

1) Rodents

a) Manipulanda
- Chew Toys (rats): e.g. Nylabone®, wood blocks
- Nesting material (mice): e.g. Alpha-twist, Isoblox®, Nestlets® (not for hairless mice)
- (rats): Alpha-twist

b) Nutritional
- Food: N/A

c) Socialization/Environmental
- Group housing

2) Rabbits

a) Manipulanda
- Small hard plastic balls
- Metal rings on a chain
- Plastic dumbbell
- Hardwood blocks

b) Nutritional
- Alfalfa cubes

- Greens
- Carrots
- Low sugar cereal

c) Socialization/Environmental
- Petting and grooming

3) Pigs

a) Manipulanda
- Large, hard plastic balls
- Suspended chain
- Mirror

b) Nutritional
- Food: fruit, cereals, marshmallows

c) Socialization/Environmental
- Pair housing in room when possible
- Petting and grooming
- Contact bedding (pine shavings) with small treats for foraging
- Scratching board

4) Guinea Pigs

a) Manipulanda
- Hardwood blocks

b) Nutritional
- Alfalfa cubes
- Vegetables
- Calorie Free Treats for guinea pigs (e.g. from Bio-Serv, Inc.)
- Low sugar cereal

c) Socialization/Environmental
- Group or Pair-Housing

S. Mouse Tail Biopsy

Tissue for genetic analysis of mice may be obtained by tail biopsy (tail snip) when scientifically justified and approved by the IACUC.

The following guidelines have been approved by the IACUC for the collection of mouse tail tissue. Note: tail biopsy must be described in the protocol/amendment and any proposed deviations from these guidelines require additional scientific justification

1. The genotype of a mouse is typically determined by Polymerase Chain Reaction (PCR) or Southern Blot analysis.
   - PCR analysis requires a minimal amount of tissue which can be obtained from tail biopsy. PCR provides genotyping results quickly and cheaply allowing for efficient colony management.
   - Southern Blot analysis requires larger amounts of DNA which is typically obtained by the excision of the distal tail.

2. The tail is composed of bone, cartilage, blood vessels, nerves and skin. The extent of mature vertebrae is related to the age of animals and the location along the length of the tail. A tail biopsy (2-5 mm at the distal end of the tail) that severs coccygeal vertebrae prior to completion of mineralization, which occurs when the mouse reaches 3 weeks of age, causes only minimal pain.
    - Tail amputation in mice >3 weeks of age may be a painful procedure with the potential to produce significant hemorrhage and will require anesthesia or analgesics, as well as, a scientific justification supported by a literature search for alternatives which are less invasive and/or painful.
    - A mouse's tail is important physiologically and behaviorally. Minimizing the amount of tail tissue removed will benefit the animal and its use in research.

Procedure

1. Limit the amount of tail to be amputated to 2-5 mm; 2 mm would be preferable and will minimize cutting bone. If an additional testing is anticipated, section the original tissue and freeze a segment. A second biopsy is permissible but must be done under anesthesia (see #5).
2. Gently restrain the mouse.
3. Obtain tail biopsies, using clean procedures, by cutting the tip of the tail perpendicular to the long axis with very sharp scissors. Alternatively, use a scalpel or razor blade.
4. Assure hemostasis. In mice <3 weeks, hemostasis is easily achieved by light, direct digital pressure around the tip of the tail. When necessary, hemorrhage can be controlled by cautery; a medical-grade, non-toxic, styptic powder (Kwik Stop®) or surgical adhesives. Consult the veterinarians if problems with hemostasis are encountered or expected (e.g., mutant mice with clotting disorders).
5. If required, use a short acting inhalant anesthetic, such as Isoflurane: an open-drop technique, conducted in a fume hood while avoiding direct contact with the animal, would be acceptable. Closely monitor the animal's recovery from anesthesia, which should be transient, and avoid co-housing sedated and active animals.

T. Rodent Toe Clipping for Biopsy and Genotyping

This protocol outlines a set of guidelines for the use of toe clipping as an alternate method for rodent identification and biopsy for genotyping.

1. General

a. This method should only be used when other identification methods (e.g. ear notching, tattooing, ear tags or microchip transponders.) are not feasible. This method is typically restricted to situations where young neonates need to be identified.

b. This method is covered in the "Guide for the Care and Use of Laboratory Animals" and will follow the guidelines put forth.

c. The use of this method must be outlined on the protocol and submitted to the IACUC for review. The IACUC will require justification for the use of this method over other methods.

d. Toe clipping involves the removal of the last phalageal (toe) bone of the digit, excluding the pollex. This method should only be performed by well trained personal, using a sharp, clean instrument. The removal of the distal phalange could interfere

with research testing, although there is evidence that grip strength is not compromised and that the procedure did not cause hyperalgesia at the amputation stump .e.g. In addition, neonates with clipped digits did not suffer rejection by their mothers.

e. If at all possible, genotyping should be completed at the same times as this procedure, and in fact should provide adequate tissue for the PCR genotyping.

2. Procedure

a. This procedure does not require anesthesia when restricted to neonatal rodents, up to seven days of age. Toe clipping of animals older than seven days is discouraged.

b. The cut should remove only the distal portion of the toe but should include the entire nail bed

c. Minimize the number of toes amputated. By policy, no more than two toes on one foot should be clipped and typically a numbering system that includes no more than two feet should be used.

d. Use a very sharp, clean microsurgery scissors. The instrument should be cleaned in between each animal with 70% alcohol and chlorhexidine.

e. The cut should remove only the distal portion of the toe but should include the entire nail bed.

f. Bleeding should not be a problem, but if it occurs, use gentle pressure with clean gauze.

U. <u>Separating and Weaning Rodents</u>

1. **<u>Overview</u>**:

The objective of this policy is to inform CAF and research personnel of the system of identifying overcrowded cages and newly split cages of rodents. Breeding cages must be identified with a unique number or code that can be used by CAF staff in identifying these cages and will follow the rodents when weaning pups or when dividing cages. Central Research Facilities must comply with all governmental regulations and guidelines. These guidelines are based on performance indices related to animal well-being and research with due consideration of the Animal Welfare Regulations and PHS Policy set forth by the most current edition of the NRC *Guide for the Care and Use of Laboratory Animals*.

On detection of an overcrowded cage, the PI/Lab will be contacted. At that point, the overcrowded cage must be separated **within 24 hours**. If the PI/Lab does not rectify the problem within a 24 hour period, the CAF staff will separate the animals and the cost center **will be charged a processing fee in addition to the per diem fee.**

Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

2. **<u>Details of Procedures</u>:**

a. When weaning litters, separate males and females. Follow the space requirements in ORA-CAF Animal Care 20, to prevent overcrowding of cages.

b. When writing out cards, use the original codes that are on the breeder's cage. Normally these codes can be found on the top of the parent's cage cards. For example:

When weaning pups from Cage #167, Pair 13-OB
Mark the top of the pup's cage with:
"From cage # 167" or "From Pair 13-OB"

This will help the PIs with their record keeping and help keep track of where the pups originated.

c. Also, on the "Date In" line of the cage card, write the **weaning date**. This will help keep track of how old the pups were when they were weaned.

d. Put a yellow **"Cage Split Notification"** card on the weaned cages with the date weaned and initials and breeder cage number. This card acts as a flag to the next CAF person to enter the room. This person will check the cages to ensure that they have enough food and water and that the number of animals indicated on the card is correct.

e. If a cage has too many mice, especially at weaning time and/or with multiple litters, it may be flagged by CAF or Veterinary Services (VS) staff with a "Cage Overcrowded" card. The top of the card will be filled out and the breeding cage code noted. Also, 2 small "Cage Split Notification" cards will have the breeding cage number noted on them and will be placed with the overcrowded card.

f. The researcher responsible for the flagged cage will write initials and date as the separator on all yellow cards The CAF or VS staff will check the cages during the next room check and sign off that the cages have been correctly fed and watered and that the number of mice match what is written on the card. The cards are left on the cage for the CAF Supervisor to remove and file.

3. **Recommended Practices:**

a. Pregnant females should be separated prior to **parturition** if the litter will create an overcrowded cage. When the litter is born, the cage is overcrowded, is non-compliant and needs to be rectified immediately.

b. Males should be removed to prevent a second mating if the female has a litter. If the female becomes pregnant in addition to the current litter, culling[i] or separating will be necessary if and when the second litter is born.

c. Breeding animals will require more space, particularly if neonatal animals will be raised together with their mother or as a breeding group until weaning age. Other

considerations may include culling one of the litters or separation of litters from the breeding group to allow for the safety and well-being of the breeding group.

Please contact the CAF Supervisor if you have any questions.

V. Social Housing

Social housing is the default method of housing in all Lifespan animal facilities unless otherwise justified based on social incompatibility as a result of behavior, standard agricultural husbandry practices, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC. In general, social animals must be housed in stable pairs or groups of compatible individuals.

If single housing of animals is deemed necessary, the duration should be limited to the minimum time period necessary and, where possible, animals should be rehoused with appropriate conspecifics. When animals are singly housed, attempts should be made to facilitate visual, auditory, olfactory and protected tactile contact with compatible conspecifics as appropriate for the species.

In situations where animals are housed alone in rooms without conspecifics, additional enrichment should be offered, such as positive interaction with humans, periodic release into larger enclosures, supplemental enrichment items, and/or the addition of a companion animal in the room or housing area.

Exceptions

Social animals may need to be singly housed for a variety of reasons. The following are the known general categories of exceptions to social housing and the IACUC approval requirements for each:

a. **Social incompatibility, standard animal husbandry and management practices*:*** The IACUC approves single housing of social animals for standard agricultural husbandry practices or situations where attempts to socially house the animals could jeopardize animal welfare. When animals are singly housed for one or more of such reasons, ***specific justification in the animal use protocol and case by case approval by the IACUC is not required.*** Examples of such situations include, but are not limited to:

- separation of aggressive or incompatible conspecifics (for example adult males of certain species such as rabbits where aggression is a documented issue)
- individual housing due to attrition of cage/pen mates or uneven number of animals
- pregnant females separated to prior to or at the time of parturition to prevent overcrowding following birth of offspring
- quarantine prior to entering or reentering a facility or herd
- separation of littermates at weaning when the number of offspring does not allow for all animals in a litter to be placed with a compatible cage mate (for example, single male weanlings)
- animals housed singly for short term recovery post-operatively; single housing must be for the minimum amount of time post-operatively necessary for recovery and/or healing as determined by the PI in consultation with the Animal Care veterinarians

- individual housing when an animal is considered a danger to other animals, to itself or personnel

b. **Clinical Necessity:** Veterinary staff may require individual housing of animals due to medical concerns. In such cases, **IACUC approval is not required.** The responsible Veterinarian will record the period of single housing and the frequency of reevaluation in the animals' medical record, will monitor the animal as noted and re-house the animal when the clinical concern is resolved. These cases will be reported to the IACUC at the discretion of the Attending Veterinarian.

c. **Scientific Necessity:** When the single housing of social species (other than short term recovery from experimental manipulation) is required for scientific reasons, specific justification must be described in the animal use protocol or an amendment. Social housing for scientific purposes must be reviewed and approved by the IACUC, and single housing cannot begin until **approval is granted by the IACUC for that protocol.**

VII. General Information

A. Animal Procurement

Hospital policy requires that all-vertebrate animals intended for teaching and/or research be purchased or transferred by the CRF office only. No animals will be purchased unless the Institutional Animal Care and Use Committee (IACUC) has granted prior protocol approval.

Animal orders may be placed by facsimile or email. A copy of the animal order form can be found on the Core Research Services webpage at *http://www.lifespan.org/research/administration/core-research-services*. The deadline for placing animal orders is 3:30 PM Thursday for deliveries to be made the following week.

The CRF Management must be consulted in advance of any requests for animal procurement through a non-commercial vendor. The CRF makes an effort to use vendors who maintain strict animal health programs that include monitoring for infectious agents by serologic and other diagnostic procedures. Also, the Attending Veterinarian must be consulted for new vendor requests. In general, the CRF tries to avoid mixing animals from sources, which might have different microbiological backgrounds.

B. Conditioning Period

The conditioning periods required for incoming animals are dependent on the species, the vendor/source of the animals, and their intended use. Experimental studies indicate that all animals should be allowed seventy-two hours to acclimate to their new environment and recover from the stress of shipping. Experimental results may vary considerably in the post-shipment period. Animals may carry agents that are communicable to man and other animals. The veterinary personnel may perform various diagnostic tests dependent upon species to ensure that animals are free of such agents (*Appendix 6 Animal Health Program* contains routine tests performed by species). Animals are usually conditioned in conventional animal rooms.

Vendors supplying rodents perform in-house surveillance on their colonies. The following chart provides the conditioning periods for commonly used species.

Minimum conditioning period:

Rats/Mice	3 days
Guinea Pigs	3 days
Hamsters	3 days
Rabbits	3-7 * days
Ducks	3 days
Pigs	7 days
Cats	7 days
Dogs	7 days

*Note: rabbits being used in research with a surgical component will have a 7-day acclimatization.

Quarantining of animals received from non-conventional vendors/sources is mandatory. The animals coming from non-conventional vendors/sources must be quarantined for up to sixty days. All rodents imported from non-commercial vendors/sources to the Coro East Barrier must be rederived. See Section VII.G.

C. Animal Transfer Policy

When an Investigator has surplus animals that they wish to donate or transfer to another Investigator within Lifespan, they must use an Animal Transfer Form. This form can be obtained from the intranet through the Core Research Services webpage: *http://www.lifespan.org/research/administration/core-research-services*.

All fields must be completed – the form will be validated by the IACUC Coordinator before the transfer is accepted. The signature of the donating and receiving Investigators must be on the form.

Submit the completed and signed form to the IACUC Coordinator or the CRF main office for verification. No animals are to be transferred or used on any protocol until the IACUC Coordinator has verified the number of animals, protocol and cost center. The IACUC Coordinator will return a signed copy of the form by email indicating that the transfer has been accepted. Once the transfer has been accepted, it is the labs' responsibility to change the PI name or protocol number and cost center. The CRF will change the information in the database.

D. Quarantine (Importation) Requirements

Laboratory animal facilities are now being asked to receive rodents from many more different sources than was the case just a few years ago. Moreover, many of these are transgenic or genetically altered animals supplied by research investigators from other institutions. Although health status information is usually available to the Central Research Facilities office before animals are shipped, the confidence level that animals are free of significant murine parasites or pathogens is much lower than it is when they are purchased from reputable commercial suppliers. The trend toward sourcing from multiple non-commercial institutions will probably increase in the future. The RIH animal facilities have established the following quarantine program in order to help protect all investigators using rodents from the incursion of variables which could confound research results.

Disposition for Importing Rodents – by Risk Level

Risk Level[1]	Disposition
Approved (Commercial)[2]	Direct into Animal Room
Low Risk	60 Day Quarantine and Testing
Low to Moderate Risk	Quarantine or Rederivation at Vet's Discretion[3]
Moderate to High Risk	Requires Rederivation[3]

[1] Exporting facilities that have evidence of adventitious rodent infections either in the animal room or in close proximity will be considered moderate to high risk.

[2] These approved commercial sources maintain barrier facilities and rigorous health monitoring programs which are frequently reviewed by the veterinarians. Examples of approved commercial sources include Charles River Laboratories, Taconic, Jackson Laboratories, and Harlan.

[3] The Attending Veterinarian (AV) is available to assist the Principal Investigator in getting the animals rederived.

1. **Rodents will only be directly imported from facilities designated as low-risk.**

   - The Principal Investigator (PI) requesting to Import rodents from an unapproved (non-commercial) source is responsible for providing CRF with the necessary contact information at the Exporting facility. Forms have been developed and are available from the Quarantine Coordinator (QC).
   - The QC is responsible for contacting the Exporting facility to obtain the rodent health information and to arrange for shipment to the Quarantine Facility. The PI will be notified and requested to assist in the event the QC is experiencing difficulties in making the contact.
   - The CRF QC is responsible for coordinating receipt of the imported animals into the Quarantine Facility, notifying the PI of their receipt, obtaining progress reports of the Quarantine, and receiving the imported animals into the CRF animal facility once Quarantine is complete.

2. **Import Procedures**

   - (PI) Contact the QC to initiate the importation procedures. The QC will provide a form (Rodent Import Request) requesting contact information concerning the Exporting facility. The information requested will include:

Exporting Facility Information

- Supplying institution
- Contact (phone #, email)
- Veterinarian (phone #, email)
- Investigator
- Species/strain
- Zygosity
- Number of animals and sex
- Coat color

- Building and room number - Special requirements

- (PI) Return the Rodent Import Request form to the QC. The form can be returned electronically to spacheco@lifespan.org.
- (QC) Contact the Exporting facility to obtain pertinent health monitoring data. The typical information requested will include:
  - General description of their rodent health monitoring program
  - Panel of selected adventitious agents for testing
  - Testing schedule (routine, frequency)
  - Recent test results from room/building (viral, parasitic, and bacterial)
  - Historic (1 year) test results from room/building (viral, parasitic, and bacterial)
- (QC) Provide the PI and AVs with progress updates. Two weeks will be allotted to obtain this information. In the event of problems, including lack of response, the PI will be promptly notified in writing that QC requires additional assistance to proceed.
- (QC- AVs) Assess the Export facility's rodent health monitoring program and designate the risk level. Notify the PI and discuss the disposition for the animals. At the discretion of the AV, animals at moderate risk may be approved for shipment to the Quarantine Facility. (PI understands that a "positive" quarantine test results will preclude the release of the shipment from the Quarantine Facility.) Typically, animals from moderate to high risk facilities will need to be rederived.

  NOTE: All rodents imported to the Coro East Barrier from a non-commercial source must be rederived at a vendor/facility approved by the Attending Veterinarian.
- (QC) Provide PI with appropriate Quarantine Facility paperwork to be filled out and returned to QC.
- (QC) Obtain Purchase Order for Quarantine Facility service charges. All charges will be charged back to the PI by the CRF.
- (QC) Arrange for shipping the animals and e-mail the Exporting facility an Authorization for Shipment form. This authorization will include any discussed shipping details (some of this may be done by the Quarantine Facility receiving department):
  - Strain, coat color, number, sex
  - Animal room identification
  - Common carrier
  - Shipping lading number
  - Date of shipment and receipt
  - Special requirements

3. <u>Receipt Procedures (Quarantine Facility procedures)</u>

- Each Approved Quarantine Facility has their approved own Receipt Procedures.
- If breeding is required in Quarantine, PIs may provide instructions for pairing or otherwise housing the animals.

- The QC or Quarantine Facility Manager will notify the PI of the receipt including the specific caging arrangements (sex and coat color) and other remarkable findings.
- Imported animals are under Quarantine conditions until released.

4. <u>Receipt of Animals from the Quarantine Facility into Lifespan CRF</u>

- The QC will coordinate the shipping of the Imported animals from the Quarantine Facility into Lifespan CRF animal facilities.
- The QC will notify the PI and the AV of the status of the shipment and delivery date. If the quarantine test results are "positive," this will preclude the release of the shipment from the Quarantine Facility. The AV is available to assist the PI in getting the animals rederived.
- The QC will notify the PI and AV when the shipment has arrived so they may be inspected.

5. <u>Records, Forms and Reports:</u>

- Rodent Import Request (from PI to QC)
- Rodent Importation Procedures (from QC to PI)
- Rodent Donation and Health Report Request Form (from QC to Exporting Facility)
- Progress and Status Reports (from QC to PI)
- Health Reports (from Exporting Facility to QC and AV)
- Quarantine Facility Services Request forms (QC to PI to QC to Quarantine Facility)
- Authorization for Shipment (from QC to Exporting Facility, AV, and PI)

6. <u>Resources for Quarantine:</u>

- Brown University Quarantine

** Note: shipments will be scheduled into Brown Quarantine on a "space available" basis

- Charles River Laboratories
- Harlan
- Taconic

E. <u>Transportation of Animals</u>

1. Between Buildings on Campus:

Animals must be conveyed in appropriate transport cages when moving within or between buildings or laboratories. No animals are to be moved without proper containment. Rodent cages must have micro isolator tops in place while being transported. All cages must be covered during transport using a towel, surgical drape or another opaque material. The CRF has a limited supply of transparent cages available for short term loan. Transportation devices should provide safety, adequate ventilation for the animals and should be able to withstand sanitation procedures. "Veri kennels" are provided for larger animals. <u>Animals transported from the animal facility cannot be housed in research laboratories or procedure rooms overnight.</u>

Used transport cages and "Veri kennels" must be returned to the facility cage wash area so the facility technical staff may properly sanitize them before reuse.

Rodents leaving the Coro East Barrier will not be allowed to return to the Barrier. They will be placed in disposable containers for transport, as cages cannot be returned to the Barrier Facility once removed.

2. Between Main Campus and Off Sites

No animals may be transported from the main campus and the off sites (and vice versa) without the express knowledge and consent of the CRF management.

3. Between Lifespan and Brown University Facilities

No animals may be transported between Lifespan and Brown University facilities without the express knowledge and consent of the CRF management.

4. Between Institutions

The CRF recognizes the need to transfer animals from one institution to another. All requests for animal transfer or receipt of animals by RIH, other than those procured through CRF purchasing services must receive approval in advance from the CRF management and the attending veterinarian. All arrangements for said shipping or receipt of animals will be processed by the CRF office. Once animal health status has been discussed between institutional veterinarians the animals will be cleared for shipping or receipt. A certificate of health signed by the veterinarian must accompany interstate shipping of animals. A USDA "record of requisition, disposition or transport of animals" form may be required and must accompany that species in transit.

5. Patient Areas

Transport of animals into patient areas needs to be authorized by the IACUC, the department head and in some cases, the Biohazard and Laboratory Safety Committee and the Department of Epidemiology, Infection Control Management.

On a rare occasion testing may be conducted on animals within a diagnostic area of the hospital. The use of diagnostic procedural areas and equipment may only be conducted with prior approval of the Department of Epidemiology and Infection Control. See *Section O below; Clinical Area Use Sanitation Procedures.*

6. Miscellaneous

Other types of transport not herein expressly mentioned will be considered on a case by case basis by the CRF management and the attending veterinarian.

F. Per Diem and Other Billable Expenses

A partial cost recovery program (per diem) for boarding and housing charges has been established. Per Diem helps cover the cost of procurement, processing paperwork, and care of animals used in research and education. Per Diem rates are reviewed and established yearly by CRF management.

The LabTracks database program tracks daily cage census and calculates monthly invoices. When animals are received, the PI, protocol, cost center and animal information are entered into the database, Barcoded cards are printed out with the PI's assigned color and detailed information. The cage is assigned the per diem rate in the database. It is critical to return the card to the CAF Supervisor after euthanasia so the cage can be removed from the system and stop charging per diems. Per Diem rates can be requested from the CRF office or found on the Intranet, Research Administration CRF page at *http://www.lifespan.org/research/administration/core-research-services*.

G. Identification of Animals

Animals must be clearly identified at all times with cage cards bearing the standard information (*Appendix 7 Cage Card*).

CRF personnel prepare cage cards when the animals are received into the facilities. However, any investigator subdividing animals or otherwise altering cage arrangements must complete all data requested on each new cage card. CRF Staff will enter the new cage information into the LabTracks database and print out new cards with the barcode. An investigator may add data to the card, as desired, but the basic information must be legible. All investigators have color-coded cage cards assigned to them.

If animals will not be returning, the cage card needs to be initialed and dated under euthanasia and the card placed on the clipboard in the respective animal room. If only one of several animals will be taken, subtract one from the number on the cage card, initial and date. Cage cards must never be discarded. If an entire rack of animals will not be returning, please notify the CRF Supervisor.

USDA covered animals must carry individual numbers either as a tattoo or ear tag. Cage cards for chronic animals should be kept with the animal(s) at all times.

Please notify the CRF office immediately if cage cards are missing.

H. Husbandry

1. Food

Natural ingredient diets are utilized in the animal care facilities. These diets are manufactured in environments which do not handle pesticides, insecticides, growth promoters, antibiotics, etc., using closely controlled processing techniques to ensure consistent nutrient content; the approximate nutritional compositions are provided. All shipments are monitored for the date of manufacture. All diets are utilized within 180 days of milling. All feed within a shipment is checked for damage or improper packaging and refused if unsatisfactory. All feed bags are sprayed with a germicidal compound prior to being placed in the feed room. Rodent diets are purchased irradiated or, for the Coro East Barrier, autoclavable diets are sterilized in the facility. Please contact the CRF Office for a list of specific diets used within the facility.

If investigators require food of the same milling lot for the duration of their studies, CAF staff should be consulted in advance. Specialized diets, including semi-purified and chemically defined diets, are available from several vendors. The CRF office can be consulted for details.

Animals are fed daily by Animal Care Technicians except for special diets.
Note: Any special diets are to be acquired and dispensed by individual laboratories unless special arrangements have been made with the CRF.

2. Water

All laboratory animals are provided with tap water (except in the Coro East Barrier). Automatic watering systems are available for large animal housing pens. No bottles, stoppers, sipper tubes, waterers, or bowls are re-used before being properly sanitized. Water bottles are changed a minimum of once per week.

The Coro Barrier automatic watering system provides reverse osmosis water which is chlorinated to 2.0 to 4.0 ppm. This water is provided through valves at each cage in ventilated racks. The same high quality water can be provided in bottles as needed.

Water is available to the animals at all times – exceptions must receive IACUC approval.

3. Environmental Conditions

The light / dark cycles in the animal rooms are 12 hr / 12 hr: 7:00 AM – 7:00 PM in Middle House, and 6:00 AM – 6:00 PM in Coro East and West, , unless noted otherwise. Timers do allow for other time cycles. PIs requesting accommodations for light sensitive studies should contact CRF management.

The relative humidity target in the animal rooms is 30-70 %. See the list of species below for specific room temperatures. All personnel should be aware that the rodent cage temperature and humidity for ventilated and non-ventilated cages may differ.

The generation of noise and vibration from humans and machinery is minimized as much as possible. Loud animal species are housed away from quieter ones. The animal rooms are remotely situated from the cage wash areas in all CRF animal facilities. Voices must be kept to a minimum in the animal rooms. Unless prior approval has been granted by the AWC, music may not be played in the animal rooms. Noisy cart casters must be repaired or replaced.

4. Animal Care by Species

- **Mice / Hamsters / Gerbils**
  Room Temperature: 70-74 °F (recommended range 68-79 °F)
  Feed: Dry ration provided in wire lid feeders ad libitum.
  Caging: Group housed in shoebox cages *or individually if justified*.
  Bedding: Corn cob with nesting material
  Cages in ventilated racks changed weekly. Static cages changed 2 times weekly.

- **Rats**
  Room Temperature: 70 -74°F (recommended range 68-79 °F)
  Feed: Dry ration provided in a wire lid feeder ad libitum.
  Caging: Group housed in shoebox cages *or individually if justified*. Some may be housed in suspended wire cages or metabolic cages due to experimental design.
  Bedding: Corn cob with enrichment
  Cages changed 2-3 times weekly

- **Guinea Pigs**
  Room Temperature: 70-74 °F (recommended range 68-79 °F)
  Feed: Vitamin C enriched diet provided in a stainless steel bowl ad libitum.
  Caging: Group housed in stainless steel rabbit cages with the suspended floor removed. Some may be individually housed in shoebox cages due to experimental design.
  Bedding: Corn cob
  Bedding changed 3 times per week.

- **Rabbits**
  Room Temperature: 66 -70°F (recommended range 61-72 °F)
  Feed: Rabbit diet provided in a stainless steel J feeder and loose timothy hay placed on the floor of the cage once per day in the morning.
  Caging: Group housed in compatible pairs or groups in suspended stainless steel cages with either stainless grated flooring or plastic, or floor housed in pens. *Individual housing in cages if justified.*
  Bedding: Plastic lined paper pads are placed in the pans under the flooring. Liners replaced 3 times a week. Wood shavings as contact bedding if housed in pens.

- **Pigs**
  Room Temperature: 70-74 °F for adults over 15 Kg, 74-78 for pigs under 15Kg (recommended range 61-81 °F)
  Feed: Dry ration provided in stainless steel J feeders or bowl once per day in the morning.
  Caging: Group housed in a room with stainless steel lower walls containing pens divided by a chain-link fence. *Individual housing if justified.*
  Bedding: Wood shavings. Post-operative animals may be recovered on raised floor grates.
  Soiled bedding removed daily.

- **Ducks**
  Room Temperature: 64-68 °F (recommended range 61-81 °F)
  Ducklings: 80 – 85 ^{o}F, drop by 6 ^{o}F each week. (Agricultural Guide, page 44.)
  Feed: Waterfowl diet provided in heavy gauge plastic fowl feeders ad libitum.
  Caging: Group housed in a pen with a steel corral and a swimming pool with a non-slip steel access ramp.
  Bedding: Wood shavings.
  Soiled bedding removed daily.

  Room conditions and cage cleaning tasks are documented on the Room Check Log.

5. <u>Cage Cards</u>

Animals are provided with cage cards at the time of receipt. After euthanasia, these cards are returned to the CAF. Cards placed on cages to flag for problems by the CAF staff may only be removed by CAF staff.

Each rodent room has a number of instructional cage cards available to flag cages. Below is a list of the cards and meaning.

- Cage Overcrowded – Too many animals, animals must be separated
- Cage Split Notification – animals were separated, CAF to check
- CRF Available – animals transferred to CAF
- Hazards – agent used, precautions
- Health Check – medical issues noted, needs to be addressed
- H_20 – agent added to water, special instructions for changing or supplying water
- Malocclusion – overgrown teeth, trimming of teeth
- Noncompliant – missing or incorrect information listed on cage card

- Notes – reminder, notes for lab, general purpose
- NPO – do not feed, fasting (for large animals)
- Rodent NPO- do not feed and/or water (rodents)
- Please Check – issue noted, needs to be addressed
- Pregnant/DOB – animal breeding, due dates, birth dates, wean dates
- Special Food – study specific, provide supplemental items, provide wet pellets on cage floor
- Surgery Care – surgical and post-operative information
- Survival Study – study specific

7. Space Requirements

Rodents are preferably housed with more than one per cage. There are minimum space requirements for each species. A list of the appropriate number of mice, rats or rabbits by weight or age for each cage type is posted on the back of each animal room door. The space requirements for larger animals are listed in the CAF husbandry Standard Operating Procedures for each species.

Cages must not be overcrowded. Care must be taken to keep the number of breeding mice and litters appropriate for the size of the cage. If a cage is found to be overcrowded, CAF staff will notify the investigator and mark the cage. The overcrowding must be corrected within **24 hours** or a fine and other charges may be imposed. Please see *Section VI, U; Policy for Separating and Weaning Rodents*.

8. **Cage and Equipment Sanitation Policy**

All non-disposable items in the Central Animal Facilities and procedural laboratories must be made of materials that are cleanable and sanitizable by high heat (cage washer or autoclave) or by chemical disinfectants. Plastic rodent cages must be replaced if cracked or crazed. Rusty equipment must be repaired or replaced. Items made of wood need clearance by CRF management. The CRF staff performs routine inspections of all animal facility and procedural laboratory areas and may cite non-sanitizable surfaces and equipment. Corrugated cardboard boxes are not sanitizable and may not be kept in animal housing rooms or be brought into the Coro East Barrier.

I. Use of Image Capturing Devices

The use of any image producing device, as mentioned under Objectives, is **strictly prohibited** in all areas of the Central Research Facility (CRF), without prior permission from the appropriate CAF supervisor, veterinary services supervisor and CRF Director. This includes, but is not limited to; the research operating rooms, procedure rooms, animal housing rooms, research areas, research personnel, and corridors of all CAF facilities.

The following will be allowed only after permission is granted by the appropriate CRF Research OR Supervisor and CRF Management.

1. Research Operating Rooms

Only image recording deemed necessary to document surgical instrumentation, technique, product application and results pertinent to the objectives of the research project will be allowed. Permission of the OR Supervisor and CRF Management must be obtained before any recording is approved.

Image recording devices must be openly displayed to the OR staff. Recorded images will be monitored by staff and only pertinent (as specified above) images/data will be allowed to be recorded. Video recording of laparoscopic procedures within the context of acceptable practice is allowed. These procedures may only be recorded by the use of VCR adaptation within the laparoscopic equipment tower. Permission of the OR Supervisor and CRF Director must be obtained.

2. Procedure Rooms, Animal Housing Rooms and Corridors of all CAF facilities:

Image recording of research animals, research animal housing areas, research laboratories and research personnel is **strictly prohibited**. Any recording of the above mentioned areas must have the approval of the CRF Management.

J. Use of Animals in Clinical Areas- Sanitation Protocol

When animal procedures are scheduled in areas such as CT scan, MRI, or Gamma Knife, the following precautions will be followed to minimize any potential contamination of those areas:

1. Pre-Transport Equipment:

- Disinfect the stainless steel gurney used for animal transportation in the animal equipment rack washer using a quaternary detergent and 180 degree rinse water just prior to use.
- The animal transport sled is made of a hard plastic surrounded by a lip deep enough to contain any urine and/or feces. Disinfect this sled using a quaternary ammonium compound (i.e. Tech Surf II) just prior to use and line the sled with a plastic sheet and absorbent material prior to the animal being placed in it.
- Spray and wipe down the animal anesthesia equipment with a quaternary ammonium compound (i.e. Tech Surf II) just prior to transport from the animal facility
- Scavenged waste gas will be contained by an f/air canister.
- A tackle box containing supportive supplies will also be sprayed and wiped down with a quaternary ammonium compound (i.e. Tech Surf II) just prior to transport from the animal facility.

2. Transporting animals:

- Spray all the wheels of the equipment being used with a quaternary ammonium compound (i.e. Tech Surf II), and allow a contact time of ten minutes prior to leaving the animal facility.
- Just prior to leaving the animal facility, anesthetize the animal with injectable anesthetics, intubate, and place into the plastic and absorbent material-lined animal transport sled.
- Cover the animal completely by a sheet so that no part of the animal will be exposed.
- Transport the animal patient and equipment to the intended location using the tunnels and avoiding public areas as much as possible.

3. Animals in Clinical Locations:

- Place plastic sheeting on the clinical patient surface of the diagnostic equipment prior to placing the animal transport sled on that surface.
- The animal must remain in the animal transport sled for the duration of the procedure.
- Personnel must wear all PPE required by both the department and those required for working with the specific species (i.e. shoe covers, procedure gowns, lab coats, etc.).

4. Post-Procedure:

- Remove the animal transport sled (containing the animal) from the diagnostic unit and place it back onto the gurney.
- Remove the plastic sheeting from the patient surface of the diagnostic equipment, place into a red biohazard bag and tie.
- Sanitize the patient surface using a hospital approved disinfectant provided by the department of Environmental Services.
- Research personnel will comply with all requirements of the host department in the sanitization and restoration of the area to acceptable conditions for human use.
- Prepare the animal for transport back to the animal facility in the same manner in which it was prepared for transport to the clinical location (i.e. anesthetized with injectable anesthetics, intubated, and completely covered by a sheet so that no part of the animal will be exposed.
- The route back to the animal facility will be the reverse of the original route.

K. Policy on the Review of Animal Cadavers or Animal Parts Used in Research

Background

The United States Department of Agriculture (USDA), in agreement with the Office of Laboratory Animal Welfare (OLAW) at the National Institutes of Health (NIH), suggests that each institution formulate a policy on how the IACUC manages use of animal cadaver tissue and/or recognizable parts.

Strictly speaking, IACUC review is not required for the use of animal cadaver tissue in research. While the Animal Welfare Act (AWA) defines "animal" as "any live or dead animal…intended for use, for research, testing, [or] experimentation," it (and 9 CFR [part 1.1 and part 2.30 (a) (1)]) also defines a "research facility" as an entity that "uses or intends to use live animals." In addition, the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals is only applicable to activities involving live vertebrate animals. Accordingly, USDA and OLAW have agreed that formal protocol review requirements do not apply to dead animals in the research setting.

Although there is no legal mandate to provide IACUC protocol review of the use of animal cadaver tissue, it is recognized as best practice to document a review of this kind of research at the institutional level. Review provides assurance that appropriate standards have been met regarding the acquisition, use, and disposal of the cadaver/animal parts. Providing standardized overview for this kind of research also serves the best interests of the institution

for a variety of other regulatory and non-regulatory reasons (*e.g.*, biosafety, public relations, liability, occupational health and safety, etc.).

Policy

The use of cadaver tissue or animal parts for research and/or teaching must be reviewed by a program veterinarian ***where the animal carcass or tissue is being brought onto campus without prior Lifespan IACUC review***. Such sources may include, but are not limited to, slaughterhouses; other academic, private-industry, government research facilities, or commercial vendors.

Notification and review will be via submission of the IACUC's Animal Cadaver and/or Animal Parts Form. The form will be reviewed by a program veterinarian and will be filed with the IACUC Coordinator and Central Research Facility veterinary staff.

It is expected that all animal cadavers or parts obtained under this policy will meet the following requirements:

1. The animal will have been ordered, used and euthanized in accordance with all applicable regulations at its source institution including IACUC review if applicable.
2. The cadaver or animal tissues will not represent a hazard to those handling the tissues (this includes but is not limited to chemical, biological and radioactive hazards).
3. The cadaver or tissues will be disposed of in accordance with all federal, state, local and institutional regulations and policies.

L. **Use of Non-pharmaceutical Grade Sodium Pentobarbital.**

Sodium pentobarbital is a popular injectable anesthetic in rodents and other small mammalian species. It was once marketed as a veterinary anesthetic but is no longer available. The availability of pharmaceutical grade pentobarbital (Nembutal) is unreliable and inconsistent, rendering it essentially unavailable for use in animal research. While there are suitable alternative anesthetics available, for certain applications and certain studies, scientific necessity requires continued use of this barbiturate; in those cases the IACUC has the authority to approve the use of non-pharmaceutical-grade compounds due to non-availability and has established a Policy for the use of non-pharmaceutical grade materials under the following conditions:

- Scientifically necessary,
- Appropriately justified,
- Prepared from a reagent or analytical-grade powder; properly prepared by your pharmacist or other knowledgeable individual (e.g., chemist, veterinarian, researcher), with assurance of appropriate storage and handling, and
- Approval by the IACUC. In making its decision the IACUC must consider the side effects, stability, storage requirements and other considerations associated with the preparation of this agent (the PI must provide this information for IACUC consideration).

Policy
The investigator, in consultation with the veterinarian, must provide a scientific justification for the use of non-pharmaceutical grade sodium pentobarbital including why alternative pharmaceutical-grade anesthetics are not appropriate for the scientific objectives of the study.

In order to use non-pharmaceutical grade pentobarbital sodium, it must be prepared in a sterile manner, controlled for pyrogen levels and *used within 7 days of preparation*. Both the powder and diluted solutions must be stored and documented according to the hospital controlled substances policy.

Standard Operating Procedure (SOP)
Preparation of Sodium Pentobarbital injection for use as an anesthetic agent (survival or non-survival procedures):

When drugs or chemicals are formulated for injection, they must be prepared in a sterile manner. Sterile constituents (e.g. sterile drug or chemical, sterile diluents, etc.), a sterile container, and a means of keeping the preparation sterile are required. Sterile injection vials are preferred (e.g., Hospera Sterile Empty Vials) as they make it easier to visualize the formulation, and to load a syringe and allow removal of solution without exposing the contents to contaminants.

Diluents or vehicles should be from the list of acceptable components (below). Exceptions to the approved list must be included in the protocol in order to be evaluated by the IACUC on a case-by-case basis.

Containers must be labeled with a description of the drug, concentration of the formulation (mg/mL), date of preparation, and the date of expiration.

Prepared solutions must be passed through a filter (0.22μ or finer) at the time of preparation. This can be done in the process of transfer to an injection vial. If there is any question about the sterility of a prepared or stored solution, it must be discarded.

Drug solutions prepared and stored properly in a suitable injection vial must be used within 7 days of preparation. Prepare only as much as can be used in a reasonable period of time. Drugs must be stored properly. Solutions must not be used if they are cloudy, discolored, precipitated, or have become otherwise altered in appearance since initial preparation. Any solution remaining after 7 days must be disposed of per hospital pharmacy policy.

Expired drug disposal:
Expired drug containers must be labeled "*expired—awaiting disposal—do not use in animals*" and stored separately from drugs in use. Controlled substances cannot be discarded without appropriate authorization.

Formulation Procedures:
Pyrogenicity is a potential experimental variable that researchers must be aware of before injecting fluids in to animals, including the formulation of non-pharmaceutical grade drugs. Pyrogens, such as endotoxin, may cause fever when injected into an animal. Sterility by filtering does not ensure that pyrogens are not present. The core temperature of animals anesthetized with non-USP pentobarbital sodium solution should be monitored for increases potentially due to pyrogens.

Acceptable solvents/diluents/vehicles:

1. Propylene glycol USP

2. Ethyl alcohol USP

3. Water for injection USP

Recipe for 10 mL sodium pentobarbital (10mg/mL*)

Ingredients

• 100 mg pentobarbital sodium USP, Reagent or analytical grade may be substituted if USP is unavailable. Purity must be > 97%
• 1 mL ethanol (95%) USP
• 9 mL water for injection USP
• Sodium hydroxide USP or hydrochloric acid USP as required bringing pH to ~ 9.5

Mixing
The mixing process should be performed in a certified Class II biosafety cabinet. Masks, gloves, and lab coats are to be worn during preparation.

- Dissolve the sodium pentobarbital salt in 10% ethanol in water for injection USP, using autoclaved and pyrogen free glassware, and mix thoroughly.
- Adjust pH as required.
- Draw the formulation into a sterile syringe and transfer into a sterile capped vial (equivalent to a Sterile Empty Vial – Hospera) through a sterile Millex 0.22 µm filter.
- Label the vial with description of the drug, manufacturer's lot number of the sodium pentobarbital, concentration, date of preparation, and expiration date: assign 7 day expiration.

* 10 mg/mL is a suitable concentration for dosing rats and the solution should be reduced to 5 mg/mL for mice.

Notes

- Use must be recorded as a Schedule II controlled substance.
- Recipe may be proportionally adjusted to produce smaller quantities of final product

M. **Use of Avian Embryos.**

All use of vertebrate animals in research, teaching and testing is regulated by the Institutional Animal Care and Use Committee (IACUC).

Avian embryos are not considered live animals by U.S. regulatory agencies and many universities do not regulate their use in research.[1] Nonetheless, there is a consensus in the scientific community that avian embryos that have attained > 50% incubation have developed a neural tube sufficient for pain perception.[2] Also, if avian embryos hatch, intentionally or unintentionally, they are live vertebrate animals and thus, are regulated by the IACUC.

Consequently, the Lifespan IACUC has adopted the following guidelines. These guidelines were developed based on recommendations of the Institute for Lab Animal Research (ILAR)[3] and the AVMA Guidelines for the Euthanasia of Animals: 2013 edition.[2] Chicken embryos, which hatch after approximately 21 days of incubation, are considered the model species. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching.

1) Investigators using avian embryos must inform the IACUC by means of the **"Notice of Intent to Use Avian Embryos"** form (see Appendix 11). If embryos will be sacrificed prior to 3 days before hatching (i.e. day $\leq$18), the research is not subject to IACUC review unless specifically requested by the investigator. **Studies using embryos within three days of hatching (i.e. day $\geq$ 19), or using hatchlings, must be reviewed by the normal IACUC procedure for vertebrate animals.**

2) Chicken embryos **younger than embryonic day 10 (E10)** are assumed to be unable to experience pain. It is recommended that E10 or younger embryos be euthanized by hypothermia, typically by placing the eggs in a −20°C freezer for a minimum of 4 hours.

3) Chicken embryos from **E11 to E18** may perceive pain and therefore should be euthanized by rapid decapitation. Additional humane methods of euthanasia may be considered.[2]

4) Chicken embryos **E19 and older** must be euthanized by CO_2, decapitation or prolonged exposure to anesthetic agents through the air cell. Avian embryos are resistant to CO_2. Therefore, embryonated eggs must be exposed to 90% CO_2 for a minimum of 20 minutes. Dry ice is unacceptable as a source of CO_2 for euthanasia.

5) The IACUC recognizes that inadvertent hatching may occur. Investigators are asked to describe their methods for humane euthanasia of hatchlings.

References:

1. OLAW FAQ. http://grants.nih.gov/grants/olaw/faqs.htm#App_4 (accessed November 4, 2014).
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. AVMA: Schaumburg, IL.

3. ILAR. 1991. ILAR News 33(4):68-70. Issues for Institutional Animal Care and Use Committees

N. Guidelines for Counting Animals Used in Research

Institutions are required to review and approve the use of animals in research. Tracking is essential to assure that only approved animals are used, and to fulfill federal obligations for reporting animal use and ensure compliance with IACUC-approved protocols. This policy defines the Lifespan Animal Care and Use Committee's position as to which animals must be counted, and when counting must be performed.

Each IACUC protocol is approved with sufficient animals to achieve the project's scientific goals. The Principal Investigator must count and account for all animals used in association with a given protocol, and report those numbers to the IACUC during the annual and three year *de novo* review processes, for AAALAC reporting purposes, and when otherwise requested.

What must be counted?
All animals used in association with each approved protocol must be counted. This holds for research, testing, teaching, and holding protocols. Animals are reported as either:

- **Adults** – Defined as aged beyond weaning and/or able to reproduce.
- **Neonates** – Defined as young animals not yet weaned, requiring parental protection or nursing.
- **Embryos/fetal animals** – Defined in mammals as the period from implantation to birth. ***Note: Embryos/fetal animals are counted only if they are manipulated before birth.***

  Note: Avian embryos (e.g. fertilized chicken eggs) are not considered live animals by U.S. regulatory agencies and the Lifespan IACUC does not require full protocol review and approval before use, rather Notification of Use of Avian Embryos (see Avian Embryo Use Policy*).*

When should counting occur?
Animals are counted upon receipt by CRF after purchase or importation; when born as part of a breeding program, and; when manipulated as part of a protocol involving *in utero* procedures.

- **Animals purchased from a vendor or imported from outside institution:** Each animal is counted as 'used' upon arrival at the research facility. *(Example: 10 female rats with day 3 litters are received for a study on lactation following parturition. Mammary gland tissue from the adult females is studied, while the pups are euthanized. All adult females and their pups must be counted.)*
- **Animals generated via in-house breeding colonies:** All animals produced (breeders and offspring) as part of a breeding program are counted at birth, even if only a subset of those animals are eventually used for actual experimentation. *(Example: 20 mice*

are produced from a selected mating, but genotyping reveals only 5 possess the correct genotype for the research project. All 20 mice must be counted.)

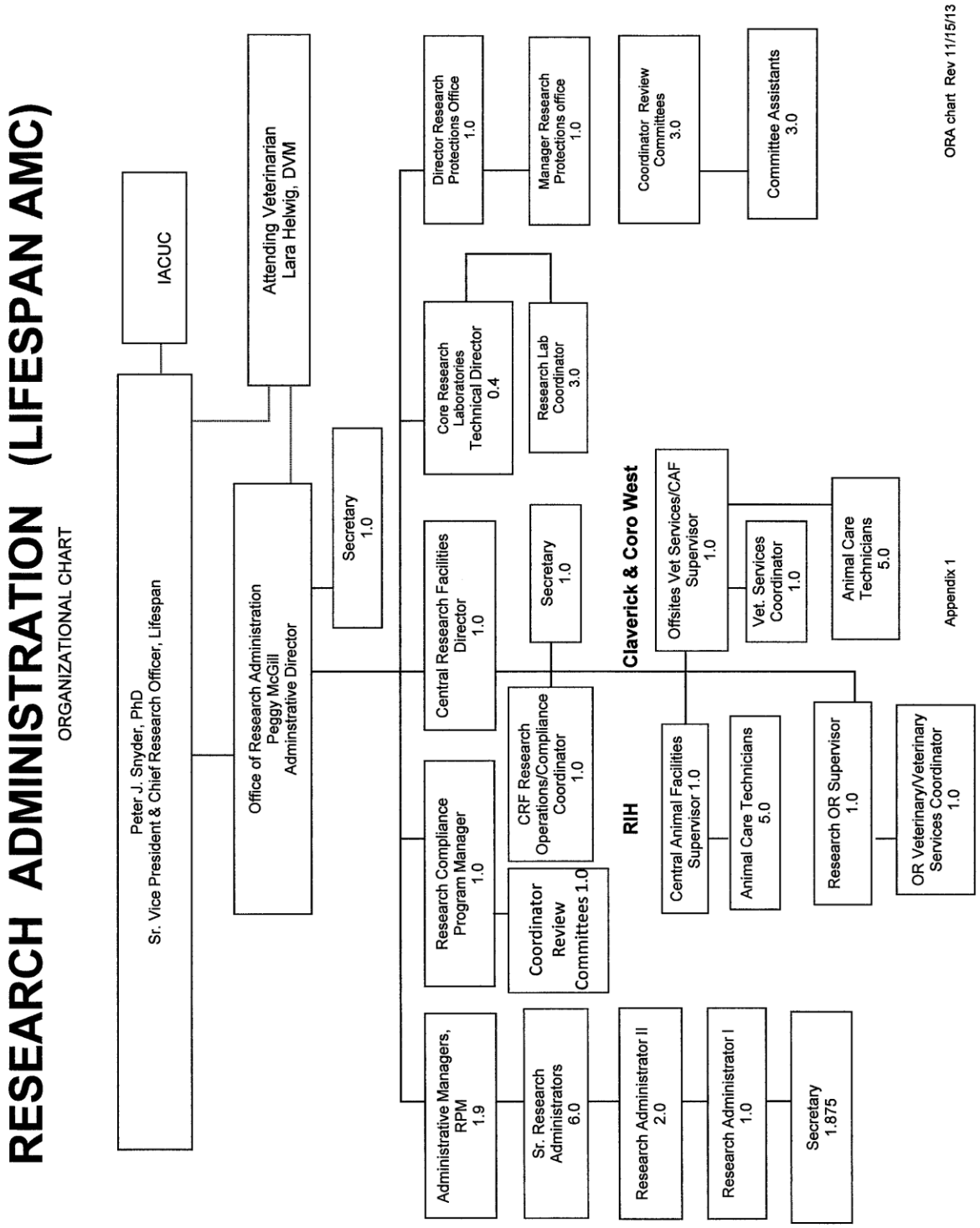
- **Animals subjected to embryonic/fetal manipulation:** Fetal animals and embryos must be counted as 'used' if they are subject to experimental manipulation prior to birth. Where there is pre-term manipulation, all animals in the litter are counted as used. *(Example: Extraction of the uterus revealed 8 embryonic pups. Only 3 were needed for the research. All 8 embryonic pups should be counted.)*

CRF Policy & Procedure Manual

Appendices

1. ORA Organizational chart
2. Zooonosis of Concern in Animal Care Facilities
3. Selection and Use of Anesthesia and Analgesia
4. Guidelines for Rodent Survival Surgery
5. Post-Op Animal Treatment Form
6. Post-Procedural Animal Treatment Form
7. Animal Health Program
8. Cage Card Sample
9. Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols
10. Cadaver and/or Animal Parts Form
11. Tumor Monitoring Form
12. Notice of Intent to Use Avian Embryos

1. ORA Organizational chart



2. Zooonosis of Concern in Animal Care Facilities

DEFINITION - Zoonosis is a communicable disease common to man and animals.

Please refer to the CRF Policy and Procedure Manual, Section V. L. for a more detailed discussion of zoonotic disease transmission and prevention.

Common Zoonoses of Laboratory Animals

1. Several species
    a. salmonellosis

        Bacteria of the genus Salmonella can be present in any domestic or laboratory animal species. Outbreaks of animal disease characterized by diarrhea have occurred in most species, and human disease caused by transmission of the bacteria via direct contact with animals has been documented. Infection with Salmonella in humans is characterized by fever, myalgia, headache, malaise, abdominal pain, vomiting, and diarrhea. Prevention of salmonellosis is based on good personal hygiene practices. Gloves should be worn when cleaning animal cages.

    b. leptospirosis

        Several species of the genus Leptospira are capable of producing disease in humans. These bacteria are most commonly associated with wild rodents, especially rats. Swine, cattle, and dogs are also host to the infection. Bacteria are excreted in the urine and enter humans through skin or mucous membranes. Commercially-bred laboratory rodents do not harbor these organisms. Dogs and swine are vaccinated for leptospirosis. Wild rodents are eliminated from the CRF. Personal hygiene and protective clothing are important methods of control.

    c. campylobacteriosis

        Infection with bacteria of the genus Campylobacter is common in many species of domestic animals. While usually asymptomatic, the organism is capable of producing diarrheal disease in most species. Human infection is characterized by diarrhea. Direct contact with fecal material of infected animals has been implicated in transmission of the disease. Infection of humans with Campylobacter of animal origin is prevented by good hygiene practices and wearing gloves while cleaning animal cages.

    d. Wild Rodents - Hantaviruses

        Hantaviruses are rodent-borne organisms which make up a genus of the family Bunyaviridae. For some time there has been concern in this country about the possible presence of Hantaan virus (the protypical hantavirus) in laboratory rodents. Hantaan virus has been known for years as the causative agent of a sometimes fatal human illness occurring in Asia, (Korean Hemorrhagic Fever and Renal Syndrome.)

        (KHFRS). Antibody liters to Hantaan virus are often found in wild rats in port cities of the United States, although KHFRS has not been recognized in this country. This virus is not present in laboratory rodents from commercial sources, but should be looked for via serological assays whenever wild-caught rodents are to be introduced to an animal facility.

Human deaths due to acute respiratory failure associated with hantavirus infection began to be recognized in the Southwestern United States in the spring of 1993. This condition presents clinically as a rapidly progressive buildup of fluid in the lungs, and has been called Hantavirus Pulmonary Syndrome (HPS). Cases were subsequently seen in Indiana, Virginia, Florida, Rhode Island, and other states. The causative agent in these deaths is now named Sin Nombre virus: its primary rodent reservoir is considered to be the deer mouse (Peromyscus maniculatis). An HPS-like fatality in Louisiana has been attributed to a different virus, provisionally called Bayou virus. Rodents are the primary reservoir for all hantaviruses, shedding virus from saliva, urine, and feces. People acquire infection most often by inhalation of rodent excreta; person to person transmission has not been documented.

Serological surveys of wild deer mice for hantavirus antibodies in Arizona, Colorado, and New Mexico showed a prevalence rate of 30%. After the fatality occurred in Rhode Island, a 1994 serological survey was done on white-footed mice (Peromyscus leucopus) from multiple locations within the state. Of 113 mice obtained from six sites (Coventry, Jamestown, West Greenwich, Richmond-Exeter, North Kingston, South Kingstown) in the southern half of Rhode Island, 20 animals (17.7%) were seropositive for hantavirus.

As of December 1994, 98 cases of Hantavirus Pulmonary Syndrome have been recognized in 21 states, with a fatality rate of 52%. One of these individuals was working in a laboratory testing wild mice for hantavirus infection. The prevalence of hantaviral infection in wild rodents and the seriousness of HPS make it clear that unusual laboratory rodents not obtained from commercial suppliers should be tested as a routine precaution. As sensitive enzyme-linked serological assays are rarely available for unusual rodent species, the testing is often accomplished indirectly by exposing commercial laboratory rodents to the new animals for a month or so before assaying the standard rodent serum for viral antibodies. In any case, plans to house rodents not supplied by commercial venders should be discussed as early as possible with Veterinary Service and Animal Care personnel. This will not only help ensure that human health is safeguarded, but will also allow adequate husbandry arrangements to be developed for the animals in question.

2. Mice
    a. lymphocytic choriomeningitis (LCM)

        Infection with the Arenavirus which causes LCM is usually inapparent in mice. The disease can be transmitted horizontally or vertically. In utero infection leads to tolerance and persistence of the virus. Transmission to humans can occur by aerosols, direct contact, or vectors.

        The disease in humans is usually clinically inapparent, but severe cases of meningitis have been reported due to LCM. Rodent vendors maintain surveillance for LCM infection in their production stock. Wild rodents can harbor the disease and must be eliminated from the CRF.

    b. hymenolepiasis

        Infection with the cestode parasite Hymenolepsis nana occurs in mice, rats, and hamsters. This tapeworm has a direct life cycle and causes few if any complications in the animal host. Humans are infected by ingestion of materials contaminated with animal feces. Development of the cestode in the human intestines can cause abdominal pain, vomiting, and diarrhea. Rodents from reliable vendors are free of H. nana. Wild rodents are kept out of animal housing areas and feed supplies.

3. Rats
    a. rat bite fever
        Two bacterial agents, Streptobacillus moniliformis and Spirillum minus, have been implicated in the disease known as rat bite fever. The rat is an inapparent carrier of these bacteria in its nasopharynx. During the incubation period of 2 to 14 days, the bite wound, inflicted by the rat will heal without complication. The affected human then experiences flu-like symptoms which may lead to polyarthritis and endocarditis in severe cases. Mortality in untreated cases is 10%. Proper handling techniques are the major means of prevention of rat bites and the associated disease.

    b. leptospirosis
        See description under 1b.

    c. ringworm
        Rats may exhibit white, crusty lesions on the head and body. See descriptions for cats 8b.

4. Hamsters
    a. lymphocytic choriomeningitis (LCM)
        See description for mice 2a.

    b. hymenolepiasis - H. nana
        See description for mice 2b.

    c. salmonellosis
        See description under 1a.

    d. campylobacteriosis
        See description under 1c.

5. Guinea Pigs
    a. ringworm
        Guinea pigs usually have no lesions. See description for cats 8b.

    b. salmonellosis
        See description under 1a.

    c. campylobacteriosis
        See description under 1c.

6. Rabbits
    a. salmonellosis
        See description under 1a.

7. Ferrets
    a. salmonellosis
        See description under 1a.

    b. caropylobacteriosis
        See description under 1c.

8. Cats
    a. toxoplasmosis

        Toxoplasmosis is caused by infection with the single- celled sporozoan Toxoplasma gondii. The definitive host for this parasite is the cat, in which infection is usually asymptomatic. Oocysts in cat feces become infective for humans and other species after a sporulation period of about 24 hours. Persons who handle cats can be infected by direct contact with fecal material. Human exposure to the organism is widespread. Nearly one-third of tested humans have antibodies to Toxoplasma. Most infections are subclinical, but infection of the fetus during the second trimester can have serious results. Abortion, encephalitis, and mental retardation are associated with intrauterine infections in humans. Pregnant women should avoid contact with cat feces. Litter pans should be cleaned daily to prevent sporulation of the oocysts in cats' feces.

    b. ringworm - dermatomycosis

        Fungi of the genera Microsporum and Trichophyton cause superficial infections of the skin and nails. These infections are common in cats, dogs, cattle, and rodents. Most human cases of ringworm are associated with exposure to infected cats. Nearly 90% of infected cats show no skin lesions. When lesions develop, they are most prevalent on the face and claws. Human ringworm is transmitted through direct contact with cats. Isolated skin lesions are treated topically.

    c. cat bite - pasteurellosis

        Most cats are normal carriers of Pasteurella multocida in their mouths. Transmission of this bacterium by a bite wound results in swelling, inflammation, and pain at the site a few hours following the incident. Prevention of cat bites through good handling techniques is recommended. Cats are not tested or treated for the carrier condition.

    d. cat scratch fever

        The etiology of cat scratch fever is Bartonella henselae. Most evidence implicates the cat as a mechanical vector for the agent rather than the host of a disease- producing organism. Seven to 20 days following a cat scratch, the human victim experiences pain and swelling at the site of the healed wound. Fever, chills, generalized pain, and vomiting may ensue. The infection is cleared spontaneously after about a week. Cat scratch fever can be prevented through the use of good animal handling techniques.

    e. salmonellosis

        See description under 1a.

    f. campylobacteriosis

        See description under 1c.

9. Dogs
    a. brucellosis

        Brucella canis infections in dogs are usually subclinical. Abortion or stillbirth of puppies may occur in infected bitches. The disease is transmitted to humans by direct contact or ingestion. Systemic infection evidenced by splenitis and lymphadenitis may occur in humans. Dogs can be tested for antibodies to B. canis by plate or tube agglutination. Disposable gloves should be worn when handling infected dogs.

    b. salmonellosis

        See description under 1a.

c. campylobacteriosis

See description under 1c.

10. Pigs

a. encephalomyocarditis

A picornavirus which primarily infects swine is the cause of encephalomyocarditis. Young pigs die suddenly due to cardiac lesions caused by the disease. Adult pigs show no symptoms. The natural reservoir of the virus is unknown, but may involve wild rodents which shed virus in feces and urine. Humans infected with encephalomyocarditis virus develop flu-like symptoms but show no evidence of cardiac pathology. No control measures for this disease are possible due to its unknown epidemiology.

b. salmonellosis

See description under 1a.

c. campylobacteriosis

See description under 1c.

11. Sheep and Goats

a. Q fever - coxiellosis

Q-Fever is a febrile (fever producing) illness caused by the rickettsia Coxiella burnetii. Humans contract the disease by inhaling infectious particles shed by ruminants (sheep, goats, cattle). While slaughterhouse workers are primarily at risk, outbreaks of Q-Fever have occurred in research laboratories; therefore care should be taken by technicians and investigators to prevent inhalation of potentially infected material at the work place.

In ruminants the disease is usually subclinical, but occasionally animals may become ill and abort their fetus(es). Likewise most human exposure goes unnoticed, although a mild flu-like illness may result. Under rare situations humans may become quite ill with prolonged fever and damage to internal organs. These cases appear to involve individuals who cannot respond appropriately to an infectious disease due to suppressed immunity or cancer. Endocarditis develops sometime after infection in a few people; however, this likelihood is greatly increased in those with prosthetic heart valves. Antibiotic treatment does not always clear the infection in people.

Diagnostic testing in ruminants does not predict with certainty whether or not animals are shedding the organism; therefore all sheep, goats or cattle should be considered potentially infected. The following general guidelines should be employed by all who handle ruminants, their excrement or fetal materials:

1. Protective outer garments should be worn to protect street clothing.
2. Masks and protective shoe coverings should be worn while in the vicinity of potentially infected animals.
3. Attempts should be made at all times to minimize the production of aerosols.
4. Careful consideration should be given at all times not to transport potentially infected equipment, supplies, etc. from the research facility to other areas.
5. Mouth pipetting, application of cosmetics, eating, smoking or drinking are forbidden in areas where potentially infected animals reside.

Individuals known to be taking drugs which suppress immunity, have artificial heart valves, or are pregnant must discuss their situation with the Veterinarian before continuing work with ruminants.

If at any time a ruminant is observed which is ill or has recently aborted, it should be immediately reported to the Veterinarian.

For further information contact the Veterinarian at 863-3223.

b. Contagious ecthyma - sheep pox

The poxvirus of sheep and goats causes epithelial proliferation and necrosis of the skin and mucous membranes of the muzzle, eyelids, oral cavity, feet, or external genitalia. The virus which is present in these lesions can be transmitted to humans by direct animal contact or through contaminated fomites. Human infection with the sheep pox virus is called orf, and is characterized by firm, large, painful nodules on the hands. Orf lesions begin as reddened areas which progress to large, weeping nodules over a 2 week period. The lesions regress without treatment in 1 to 2 months.

Prevention of orf is based on identification of the pox lesion on a sheep or goat. Animals with suspicious lesions are detected upon arrival or during quarantine and will be returned to the vendor. Persons handling sheep or goats during the quarantine period must wear disposable gloves. Development of any lesion of an animal's face, feet, or external genitalia should be reported to the veterinary staff.

c. salmonellosis

See description under 1a.

d. campylobacteriosis

See description under 1c.

12. Non-human Primates

a. Cercopithecine herpesvirus (B virus) infection

Infection with Cercopithecine herpesvirus is prevalent in wild and captured macaques. The rhesus monkey (Macaca mulatta) is the primary reservoir in the wild, but other macaques are highly susceptible to infection. Under typical monkey housing conditions, the virus spreads rapidly to infect all animals in the room. Disease in macaques is benign and usually asymptomatic, although vesicular lesions (similar to cold sores in people) may develop on the tongue and oral mucosa. Many infected macaques become lifelong carriers and intermittently shed the virus in saliva. The primary mode of transmission to humans is through bite wounds. Aerosol transmission is thought to be possible.

Cercopithecine herpesvirus infection in man has a very high mortality rate. Fewer than 10% of the individuals with known infections have survived and all of those have had permanent neurological deficits- Following one to five week incubation, the virus infects the brain and causes encephalomyelitis with no known treatment.

Prevention of human Cercopithecine herpesvirus infection requires careful handling of macaques. Disposable masks and gloves must be worn at all times when working with monkeys. Face shields are worn to guard against exposure of mucous membranes to virus in bodily fluids or secretions. General anesthesia is required to accomplish diagnostic procedures and medical treatments in most macaques.

b. tuberculosis

Non-human primates are highly susceptible to infection with Mycobacterium tuberculosis and M. bovis. They contract the disease from humans, spread it to other monkeys in the colony, and can transmit TB to humans.

Monkeys are tested for TB multiple times during quarantine and 4 times yearly with an intrapalpebral PPD inoculation. Humans working with monkeys are tested annually for TB by the Employee Health Service. Disposable mask and gloves are always worn in the presence of non-human primates.

c. shigellosis - bacillary dysentery

Non-human primates infected with bacteria of the genus Shigella can develop severe gastrointestinal disease. Some animals serve as carriers of the organisms and nay infect humans who come in contact with contaminated materials.

Rectal cultures collected during quarantine of non-human primates are screened for the presence of salmonella and shigella. Infected animals are isolated and treated with antibiotics. Disposable gloves and mask are worn when cleaning monkey cages.

d. salmonellosis

See description under 1a.

e. campylobacteriosis

See description under 1c.

Back to top

3. Selection and Use of Anesthesia and Analgesia of Laboratory Animals

I. Narcotic Agonist Analgesics

Meperidine
Buprenorphine
Butorphanol
Nalbuphine
Pentazocine

II. Narcotic Antagonists

Naloxone

III. Non-narcotic Analgesics

Carprofen
Meloxicam
Ketaprofen

IV. Tranquilizers/Sedatives

A. Phenothiazines

Acepromazine

B. Benzodiazepines

Diazepam
Midazolam

C. Alpha$_2$-adrenergic agonists

Dexmedetomidine
Xylazine

V. Alpha$_2$-adrenergic Antagonist

Atipamezole

VI. Analeptics

Doxapram

VI. Injectable Anesthetics

A. Dissociative/Alpha$_2$-adrenergic agonist combinations

Ketamine & Xylazine Combination
Ketamine & Dexmedetomidine Combination

B. Dissociative/Benzodiazepine derivative combination

Tiletamine & Zolazepam Combination

C. Ultrashort-acting Barbiturates

Sodium Thiamylal
Sodium Thiopental

D. Short-acting Barbiturates

Pentobarbital Sodium

VIII. Inhalant Anesthetics

Isoflurane

IX. Miscellaneous Anesthetics

Urethane
Tricaine Methanesulfonate

X. Dissociative Anesthetics

Ketamine

XI. Parasympathetic Blocking Agents

Atropine

Narcotic agonist analgesics differ in the clinical effects they produce due to their individual profiles of agonist/antagonist activity at mu, kappa and sigma neuroreceptors. The table below shows the neuroreceptor/effect profiles for various narcotics. Agonists promote the listed neuroreceptor effects.

Interactions of Morphine & Morphine-like Drugs with Neuroreceptors

	RECEPTOR TYPES		
	Mu	Kappa	Sigma
Effects	Supraspinal	Spinal	Dysphoria
	Analgesia	Analgesia	Hallucinations
	Respiratory	Respiratory	
	Depression	Depression	
	Euphoria	Sedation	Vasomotor
			Stimulation
	Physical	Miosis	
	Dependence		

Drugs			
Morphine	Ag	Ag	O
Buprenorphine	pAg	--	O
Nalorphine	Ant	pAg	Ag
Pentazocine	Ant	Ag	Ag
Butorphanol	O	Ag	Ag
Nalbuphine	pAg/Ant	Ag	Ag
Naloxone	Ant	Ant	Ant

Abbreviations: Ag, agonist; pAg, partial agonist; Ant, antagonist; 0, no interaction.

The table below shows the duration of action and the analgesic potency (compared with morphine) for various narcotic analgesics.

Comparison of Opioid Analgesics

Generic Name	**Trade Name**	**Dosage Index**	**Duration of Action Hour**
Morphine		1	4-5
Oxymorphone (dihydrohydroxy-morphinone)	Numorphan	7.5-10	4-5
Codeine		0.08	4-6
Hydrocodone	Hycodan	1-2	4-8

(dihydrocodeinone)

Dihydrocodeine	Percodin	0.16	4-5
Meperidine	Demerol	0.08-0.1	2-4
Buprenorphine	Buprenex	25-50	8-12

I. Narcotic Agonist Analgesics

Compound	**Species**	**Dose[1] & Route**
BUPRENORPHINE[2,a] (Buprenex) (6-12 hr duration)	Mouse	0.05-0.1 SQ
	Rat	0.01-0.05 SQ 0.1-0.25 IV
	Gerbil	------
	Hamster	------
	Guinea Pig	0.05 IM
	Rabbit	0.01-0.05 SQ, IV
	Ferret	0.01-0.03 SQ, IM,IV
	Chicken	------
	Pigeon	------
	Cat	0.005-0.01 IM, IV

	Dog	0.005-0.02 IM, IV
	Nonhuman Primate	0.005-0.01 IM, SQ 0.01 IV
	Pig	0.005-0.02 IM, IV
	Sheep	0.005-0.01 IM, IV

SC = subcutaneous; IM = intramuscular; IV= intravenous; IP = intraperitoneal; PO = per os (oral)

1. All doses are in mg/kg unless otherwise noted.

2. Rats given buprenorphine have been shown to have an increase in consumption of non-food items (pica) such as bedding. Animals should be closely monitored during post surgical recovery including food consumption, fecal production and body weight changes.

a. Laboratory Animal Anaesthesia, A Practical Introduction for Research Workers and Technicians, 2nd Edition, 1996, P Fleckness, Academic Press

Compound	**Species**	**Dose* & Route**
BUTORPHANOL (Torbugesic/Torbutrol) (2-6 hr duration)	Mouse[a]	0.2-2 SQ, IM 5.6 PO
	Rat[a]	2 SQ 2.1 PO
	Gerbil	------
	Hamster	------
	Guinea Pig	------
	Rabbit[a]	0.1-0.5 IV, SQ, IM

Ferret[a]	0.4 IM
Chicken	------
Pigeon	0.05-0.4 SQ
Raptors	0.3-1.0 SQ, IM
Cat[a]	0.4 SQ
Dog[a]	0.1 IV 0.2-0.4 IM, SQ
Nonhuman Primate	0.2 IV 0.025-0.4 IM, SQ
Pig	0.1-0.3 IM
Sheep	------

* All doses are in mg/kg unless otherwise noted

Note – Dosages of up to 4.0 mg/kg IM given every 6-12 hours have been used in birds with satisfactory results.

a. Laboratory Animal Anaesthesia, A Practical Introduction for Research Workers and Technicians, 2nd Edition, 1996, P Fleckness, Academic Press

Compound	**Species**	**Dose[1] & Route**
Morhpine[a] (4 – 6 hr duration)	Mouse	2.5 SQ
	Rat	2.5 SQ
	Gerbil	------
	Hamster	------

	Guinea Pig	2-5 SQ
	Rabbit	2-5 SQ
	Ferret	0.5-5 SQ
	Chicken	------
	Pigeon	------
	Cat	0.1 SQ
	Dog	0.5-5 SQ, IM
	Nonhuman Primate	1-2 IM, SQ
	Pig	0.2-1 IM,
	Sheep	0.2-0.5 IM

a. Laboratory Animal Anaesthesia, A Practical Introduction for Research Workers and Technicians, 2nd Edition, 1996, P Fleckness, Academic Press

II. Narcotic Antagonists

Dosages given are those to antagonize opioid respiratory effects. Naloxone is easily titrated when used intravenously since opioid reversal effects can be assessed within one to two minutes.

Compound	**Species**	**Dose* & Route**
NALOXONE (Narcan)	Mouse	2.7 SQ
	Rat	0.04 SQ

Gerbil	------
Hamster	------
Guinea Pig	------
Rabbit	0.04 SQ
	0.125 IV
Chicken	------
Pigeon	------
Cat	0.04 SQ
	0.04 IM
	0.04 IV
Dog	0.04 IM
	0.04 IV
Nonhuman Primate	0.01 IM
Pig	------
Sheep	------

* All doses are in mg/kg unless otherwise noted

Note: The half life of naloxone is 60 to 90 minutes. Redosing with naloxone is frequently necessary to prevent re-narcotization.

III. Nonsteroidal Antiinflammatory Drugs (NSAID)[1, 2]

Compound	**Species**	**Dosage and route of administration**
Carprofen (Rimadyl®)	Mouse	b 5 mg SC every 24 h, can combine with buprenorphine (0.03mg)
	Rat	b, c 5 mg SC every 24 h, can combine with buprenorphine (0.05mg)
	Gerbil	b 5 mg SC every 24 h
	Hamster	b 5 mg SC every 24 h
	Guinea Pig	b 4 mg SC every 24 h
	Rabbit	c 2.2 mg every 12 h
	Birds	b,c 1 – 2 PO, IM, IV, every 12 h, consult with veterinarian for specific recommendations
	Cat	c 2 mg SC
	Dog	a 4.0 IV, SC, IM every 12 h, decrease dose 2.2
	Nonhuman Primate	b 2 – 4 mg PO, SC every 12 h
	Pig	e 2 – 4 mg IV, SC every 12 hours
		d lambs 0.5 mg Adults 4 mg

	Sheep	
	Ferret	b 1 mg PO every 12 - 24 hours
Ketoprofen	Mouse	
	Rat	b 5 mg PO, SC every 24 h
	Gerbil	b 1 mg SC, IM every 12 - 24 h
	Hamster	b 1 mg SC, IM every 12 - 24 h
	Guinea Pig	b 1 mg SC, IM every 12 - 24 h
	Rabbit	c 1 mg IM every 12 – 24h
	Birds	b 1 – 2 PO, IM, IV, every 12 h, consult with veterinarian for specific recommendations
	Cat	a,c 2 mg SC every 24 hours decrease to 1.0 for up to 3 days
	Dog	a,c 1 – 2mg IV, SC, IM every 24 hours decrease to 1.0 up to 3 days
	Nonhuman Primate	
	Pig	
	Sheep	
	Ferret	b 1 mg PO, SC, IM every 24 hours

Meloxicam	Mouse	b 1 - 2 mg PO, SC
	Rat	b 1 - 2 mg PO, SC
	Gerbil	
	Hamster	
	Guinea Pig	
	Rabbit	
	Birds	b 0.1 – 0.2 PO, IM every 24 h, half-life 3x longer that other bird species, consult with veterinarian for specific recommendations
	Cat	a,c 0.2 PO,SC obtain veterinary assistance for multiple dosing
	Dog	a,c 0.2 PO,IV, SC every 24 hours, reduce dose 0.1 PO
	Nonhuman Primate	
	Pig	f 0.4 – 0.8 mg IM
	Sheep	
	Ferret	0.2 mg/kg SC, IM 0.3 mg/kg PO every 24 hours

SC = subcutaneous; IM = intramuscular; IV = intravenous; IP = intraperitoneal; PO = per os (oral)

1. NSAIDs are a group of compounds that share therapeutic actions including analgesia, anti-inflammatory and antipyretic capabilities. These drugs have important roles in managing acute and chronic pain. Although in general these are very safe drugs significant clinical complications for some individuals can occur and their mode of action is inhibition of cyclooxygenase (COX) enzymes.
2. Use 1 NSAID at a time and make sure of adequate and appropriate dosing.

a. Handbook of Veterinary Pain Management, 2002, Mosby, Inc. JS Gaynor and WW Muir
b. Exotic Animal Formulary, Third Edition, 2005, Elsevier Inc.
c. Veterinary Drug Handbook, 5th Edition, 2005, DC Plumb
d. Price J and Nolan AM, Analgesia of newborn lambs before castration and tail docking with rubber rings. Vet Rec (2001) Sep 15 149(11): 321-4
e. Laboratory Animal Anaesthesia, A Practical Introduction for Research Workers and Technicians, 2nd Edition, 1996, P Fleckness, Academic Press
f. Georgoulakis IE, Petridou E, Filiousis G, et al. Meloxicam as adjunctive therapy in treatment and control of porcine respiratory disease complex in growing pigs. *J Swine Health Prod* 2006; 14(5):253-257.

IV. Tranquilizers/Sedatives

A. Phenothiazines

Compound	Species	Dose* & Route
ACEPROMAZINE[a,]	Mouse	0.5 IM
	Rat	0.5 IM
	Gerbil	causes seizures
	Hamster	0.5 IM
	Guinea Pig	0.5 IM
	Rabbit	0.25-2 IM,SQ, IV
	Ferret	0.25-0.75 IM, SQ
	Chicken	------
	Pigeon	------

	Cat	1.1-2.2 SQ, IM, IV
	Dog	0.5-2.0 SQ, IM, IV
	Nonhuman Primate	0.5-1.0 IM
	Pig	0.11-0.22 SQ, IM, IV
	Sheep	0.55 IV
		0.05-0.1 IM

* All doses are in mg/kg unless otherwise noted.

a. Veterinary Drug Handbook, 5th Edition, 2005, DC Plumb

IV. Tranquilizers/Sedatives

B. Benzodiazepines

Compound	**Species**	**Dose* & Route**
DIAZEPAM[a] (Valium)	Mouse	5-6 IP
	Rat	2.5 IP
	Gerbil	5-6 IP
	Hamster	5.0 IP
	Guinea Pig	2.5 IM
		2.5-5.0 IP
	Rabbit	2-10 IM
		1-5 IV
	Chicken	0.5-2 IV, IM

	Pigeon	------
	Cat	0.3-1.0 IM 0.1-0.5 IV
	Dog	0.3-0.5 IM
		0.1-0.5 IV
	Nonhuman	0.1-0.5 IM, IV
	Primate	
	Pig	0.5-1.5 IV, IM
		0.5-8.5 IM
	Sheep	0.5-1.5 IM, IV

* All doses are in mg/kg unless otherwise noted.

Note: Specific benzodiazepine antagonists have shown significant clinical potential. Flumazenil appears to be a specific antagonist with very little agonistic action.

a. Veterinary Drug Handbook, 5th Edition, 2005, DC Plumb

IV. Tranquilizers/Sedatives

B. Benzodiazepines

Compound	**Species**	**Dose* & Route**
MIDAZOLAM[a] (Versed)	Mouse	1-2 IM
	Rat	1-2 IM
	Gerbil	1-2 IM
	Hamster	1-2 IM
	Guinea Pig	1-2 IM

	Rabbit	1-2 IM, IV
	Chicken	1-2 IM, IV
	Pigeon	1-2 IM, IV
	Cat	0.3 mixed with ketamine
	Dog	0.3 mixed with opioid
	Nonhuman	------
	Primate	
	Pig	0.1-0.4 IM
		0.4 – 0.8 PO
	Sheep	------

* All doses are in mg/kg unless otherwise noted.

Note: Specific benzodiazepine antagonists have shown significant clinical potential. Flumazenil appears to be a specific antagonist with very little agonistic action.

a. Veterinary Drug Handbook, 5th Edition, 2005, DC Plumb

IV. Tranquilizers/Sedatives

C. Alpha$_2$-adrenergic agonists

Compound	**Species**	**Dose* & Route**
DEXMEDETOMIDINE (Dexdormitor)	Mouse	0.015-0.05 SQ
	Rat	0.015-0.05 SQ
	Gerbil	0.05-0.1 SQ
	Hamster	0.05 SQ

	Guinea Pig	0.15 SQ
	Rabbit	0.05-0.25 SQ
	Chicken	------
	Ferret	0.03-0.04 IM, SQ
	Pigeon	0.025 IM
	Cat	0.025-0.075 SQ, IM
	Dog	0.005-0.04 SQ,IM,IV
	Nonhuman Primate	------
	Pig	0.04 IM
	Sheep	0.0125 IM

* All doses are in mg/kg unless otherwise noted.

IV. Tranquilizers/Sedatives

C. Alpha$_2$-adrenergic agonists

Compound	**Species**	**Dose* & Route**
XYLAZINE (Rompun)	Mouse	4-8 IM
	Rat	4-8 IM
	Gerbil	4-8 IM

Hamster	4-8 IM
Guinea Pig	3-5 IM
Rabbit	3-5 IM
	3-9 IV
Chicken	5-10 IM
Pigeon	5-10 IM
Cat	1.1-2.2 SQ, IM
	0.5-1.0 IV
Dog	1.1-2.2 SQ, IM
	0.5-1.0 IV
Nonhuman Primate	0.5-1.0 IM
Pig	2-3 IM
Sheep	0.1-0.3 IM
	0.05-0.1 IV
	0.22 SQ

* All doses are in mg/kg unless otherwise noted.

V. Alpha$_2$–adrenergic Antagonist

Compound	Species	Dose* & Route
ATIPAMEZOLE (reversal agent for Dexmedetomidine and Xylazine)	Mouse	1 SQ, IP, IV SQ, IM @ 5x dose agonist used
	Rat	1 SQ, IP, IV
	Gerbil	1 SQ, IP, IV
	Hamster	1 SQ, IP, IV
	Guinea Pig	1 SQ, IP, IV
	Rabbit	1 SQ, IP, IV
	Chicken	------
	Pigeon	------
	Cat	1 SQ, IP, IV
	Dog	1 SQ, IP, IV
	Nonhuman Primate	------
	Pig	1 SQ, IP, IV
	Sheep	1 SQ, IP, IV

* All doses are in mg/kg unless otherwise noted.

VI. Analeptics

Compound	Species	Dose* & Route
DOXAPRAM (Dopram)	Mouse	------
	Rat	------
	Gerbil	------
	Hamster	------
	Guinea Pig	------
	Rabbit	2 IV
	Chicken	------
	Pigeon	------
	Cat	5.5-11 IV
	Dog	5.5-11 IV
	Nonhuman Primate	------
	Pig	2-10 IV
	Sheep	2-10 IV

* All doses are in mg/kg unless otherwise noted.

VII. Injectable Anesthetics

A. Dissociative/Alpha$_2$–adrenergic agonist combinations

Compound	Species	Dose* & Route
KETAMINE & XYLAZINE COMBINATION	Mouse	
	Rat	40-90 IM Ketamine
		13 IM Xylazine
	Gerbil	------
	Hamster	40-150 IM Ketamine
		5-10 IM Xylazine
	Guinea Pig	35 IM Ketamine
		0.2-0.5 IM Xylazine
	Rabbit	44 IM Ketamine
		5 IM Xylazine
		10 IV Ketamine
		1.0 IV Xylazine
	Chicken	10-30 IM, IV Ketamine
		2-6 IM, IV Xylazine
	Pigeon	10-30 IM, IV Ketamine
		2-6 IM, IV Xylazine
	Cat	------
	Dog	------
	Nonhuman Primate	------

	Pig	12-20 IM Ketamine
		2.2 IM Xylazine
	Sheep	11 IM Ketamine
		0.22 IM Xylazine
		2-3 IV Ketamine
		0.22 IM Xylazine

* All doses are in mg/kg unless otherwise noted.

VII. Injectable Anesthetics

A. Dissociative/Alpha$_2$–adrenergic agonist combinations

Compound	**Species**	**Dose* & Route**
KETAMINE & DEXMEDETOMIDINE COMBINATION	Mouse	75 IP Ketamine
		0.5 IP Dexmedetomidine
	Rat	75 IP Ketamine
		0.25 IP Dexmedetomidine
	Gerbil	75 IP Ketamine
		0.25 IP Dexmedetomidine
	Hamster	100 IP Ketamine
		0.125 IP Dexmedetomidine
	Guinea Pig	40 IP Ketamine
		0.25 IP Dexmedetomidine
	Rabbit	25 IM Ketamine
		0.25 IM Dexmedetomidine

	Chicken	3-6 IM Ketamine
		0.025-0.05 IM Dexmedetomidine
	Pigeon	3-6 IM Ketamine
		0.025-0.05 IM Dexmedetomidine
	Cat	7 IM Ketamine
		0.04 IM Dexmedetomidine
	Dog	2.5-7.5 IM Ketamine
		0.02 IM Dexmedetomidine
	Nonhuman Primate	------
	Pig	10 IM Ketamine
		0.04 IM Dexmedetomidine
	Sheep	11 IM Ketamine
		0.0125 IM Dexmedetomidine

* All doses are in mg/kg unless otherwise noted.

VII. Injectable Anesthetics

B. Dissociative/Benzodiazepine derivative combination

Compound	**Species**	**Dose* & Route**
TILETAMINE & ZOLAZEPAM (Telazol)	Mouse	------
	Rat	15-40 IM
	Gerbil[a]	20 mg in combination with
		10 mg xylazine

	Hamster	50 IM
	Guinea Pig	10-140 IM
	Rabbit	3 SQ
	Chicken	------
	Pigeon	------
	Cat	2-15 IV
		2-15 I~~V~~ M
	Dog	2-15 IM
	Nonhuman Primate	1.5-16 IM
	Pig	4.0-8.8 IM
	Sheep	------

* All doses are in mg/kg unless otherwise noted.

Note – Telazol doses higher than 3 mg/kg may cause renal tubular necrosis in the rabbit. Exercise care when using Telazol in this species.

a. Veterinary Drug Handbook, 5th Edition, 2005, DC Plumb

VII. Injectable Anesthetics

C. Ultrashort-acting Barbiturates

Compound	**Species**	**Dose* & Route**
SODIUM THIOPENTAL	Mouse	25-50 IV
		50 IP
	Rat	20 IV
		20-48 IP
	Gerbil	20 IV

		40 IP
	Hamster	20 IV
		40 IP
	Guinea Pig	20 IV
		20-25 IP
	Rabbit	20-50 IV
	Chicken	13-18 IV
	Pigeon	13-18 IV
	Cat	15-25 IV
	Dog	15-25 IV
	Nonhuman Primate	22-25 IV
	Pig	5-19 IV
	Sheep	20-25 IV

* All doses are in mg/kg unless otherwise noted.

VII. Injectable Anesthetics

D. Short-acting Barbiturates

Compound	**Species**	**Dose* & Route**
PENTOBARBITAL SODIUM (Nembutal)	Mouse	40-80 IP
		40-70 IV
	Rat	10-45 IP
		10-50 IV
	Gerbil	60-90 IP, IV
	Hamster	50-90 IP

		30 IV
	Guinea Pig	30-45 IP, IV
	Rabbit	20-45 IV
	Chicken	------
	Pigeon	------
	Cat	30-35 IV
	Dog	30-35 IV
	Nonhuman Primate	20-35 IV
	Pig	20-30 IV
	Sheep	25-30 IV

* All doses are in mg/kg unless otherwise noted.

VIII. Inhalant Anesthetics

Compound	Species	Dose* & Route
ISOFLURANE (Forane)	Rat	To effect – Inhalation
	Mouse	To effect - Inhalation
	Gerbil	To effect - Inhalation
	Hamster	To effect - Inhalation
	Guinea Pig	To effect - Inhalation
	Rabbit	To effect - Inhalation

Chicken	To effect - Inhalation
Pigeon	To effect - Inhalation
Cat	To effect - Inhalation
Dog	To effect - Inhalation
Nonhuman Primate	To effect - Inhalation
Pig	To effect – Inhalation*
Sheep	To effect – Inhalation

* All doses are in mg/kg unless otherwise noted.

Table 11-10. MAC Values for a Variety of Species

	Methoxyflurane	*Halothane*	*Isoflurane*	*Enflurane*	*Sevoflurane*	*Desflurane*	*N20*
Cat	0.23[265]	*1.14*[266] 0.82[268] 1.19[269] 0.99[270]	1.63[105] 1.61[269]	1.20[265] 2.37[269]	2.58[66]	9.79[267]	255.00[266]
Dog	0.23[271] 0.24[276] 0.29[277]	0.86[272] 0.87[266'276] 0.92[278] 0.93[281] 0.89[274]	1.28[105] 1.39[274] 1.30[86]	2.20[273] 2.06[87] 2.25[279] 2.06[279]	2.36[274] 2.10[66]	7.20[275]	188.00[271] 222.00[266] 297.00[280]
Horse	0.28[346]	0.88[54]	1.31[54]	2.12[54]	2.31[282]		205.00[147]
Monkey		0.89[266] 1.15[283]	1.28[283] 1.46[86]	1.84[283] 1.97[284] 2.19[285]			200.00[266]
Mouse		0.96[284] 1.00[285]	1.35[284] 1.41[285]				275.00[285] 150.00[286]
Calf		0.76[287]					223.00[287]
Pig		0.91[288] 1.25[291] 0.94[344]	1.45[289] 2.04[56] 1.51[86] 1.55[293] 1.75[344]		2.66[290] 1.97[292]	10.00[56]	277.00[291] 162.00[293] 195.00[344]
Rabbit		0.82[294] 0.80[297] 1.39[295] 1.56[298]	2.05[295]	2.86[295]	3.70[296]	8.90[275]	
Rat	0.27[299]	1.17[299] 0.81[300] 1.11[304]	1.17[300] 1.38[304] 1.52[284]	2.17[284]	2.40[301] 2.50[40]	5.72[302] 7.10[305] 6.85[306]	136.00[303] 204.00[110] 155.00[307]

		1.10[301] 1.13[309,310] 1.03[284] 1.23[306]	1.46[306]				235.00[308] 221.00[308]
Sheep	0.26[311]	0.97[311]	1.58[311]				
Birds							
Chicken		0.85[55]					
Ducks		1.04[312]	1.30[313]				
Cranes			1.34[314]				
Pigeon			1.51[315]				154.00[315]
Hawk			1.45[315]				220.00[315]
Amazon Parrot			1.47[316]				
Cockatoo			1.44[316]				
African Gray Parrot			1.91[316]				
Miscellaneous							
Toad	0.22[317]	0.67[317]					82.20[317]
Goldfish	0.13[318]	0.76[318]					
Human	0.16[319]	0.77[319] 0.73[324] 0.74[326,327]	1.15[320]	1.68[321]	2.05[296] 1.71[325]	6.00[322] 7.25[322]	104.00[323]

IX. Miscellaneous Anesthetics

Compound	Species	Dose* & Route
URETHANE[a]	Mouse	1-2 IP g/kg
	Rat	1-2 IP g/kg
	Gerbil	1-2 IP g/kg
	Hamster	1-2 IP g/kg
	Guinea Pig	1-2 IP

		g/kg
	Rabbit	------
	Chicken	------
	Pigeon	------
	Cat	------
	Dog	------
	Nonhuman Primate	------
	Pig	------
	Sheep	------

* All doses are in mg/kg unless otherwise noted.

a. Urethane (ethyl carbamate) has been widely used for the production of long intervals of anesthesia in a number of laboratory animal species and is considered to cause minimal depression of the cardiovascular and respiratory systems. (P. Flecknell, 1996) However urethane is hepatotoxic and carcinogenic in some species and should be handled with gloves. Its use is only permitted for acute/nonsurvival procedures where its use has been justified and approved by the IACUC.

IX. Miscellaneous Anesthetics

Compound	**Species**	**Dose* & Route**
TRICAINE METHANESULFONATE (MS-222)	Frog	100 mg/liter of water immersion
	Fish	50-100 mg/liter of water immersion

Buffered MS-222 is a frequently used general anesthesia for Xenopus frogs. The MS222 solution must be made fresh daily by dissolving the powder MS222 in de-ionized water to a concentration of 500-2000mg/L (typically use 1500 mg/kg) which is buffered to a pH 7.0 using sodium bicarbonate (NaHCO3)10 – 25 mEq/L (420-1050 mg/L). Monitor the depth of anesthesia through observing for loss of the righting response and by determining loss of response to painful stimuli (foot pinch). Once the animal is anesthetized, remove it from the solution while maintaining the skin moist with saline during the surgical procedure. If the frog appears to be recovering from the anesthesia prior to wound closure additional MS222 can be dripped on the skin. Following the surgery, rinse the frog in fresh frog water to stimulate recovery and maintained in very shallow water, or otherwise keep moist use a damp cloth during recovery, to minimize potential drowning before complete consciousness is restored.

Note: The efficacy of using hypothermia in amphibians has NOT been demonstrated, although the animals become torpid. Therefore, ice or ice water is not acceptable as the sole anesthetic agent.

X. Dissociative Anesthetics

Compound	Species	Dose* & Route
KETAMINE (Ketaset)		
	Cat	5-10 IM
		1-5 IV
	Nonhuman	1-5 IV
	Primate	5-25 IM

* All doses are in mg/kg unless otherwise noted.

Note – In most species, ketamine is generally used with a tranquilizer to improve muscle relaxation and to promote a smooth recovery. Ketamine may be used alone in cats and nonhuman primates.

Back to top

4. Guidelines for Rodent Survival Surgery

This appendix includes definitions, tables of information, and references as a resource for investigators. **Please refer to the CRF Policy and Procedure Manual, Section VI.G.2. for procedural details.**

DEFINITIONS:
ASEPTIC SURGICAL PROCEDURES: Surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.
MAJOR SURGERY: Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent or significant physical or physiological impairment; and/or any procedure associated with orthopedics or extensive tissue dissection.
MINOR SURGERY: Any surgical intervention that neither penetrates and exposes a body cavity nor produces permanent or significant impairment of physical or physiologic function. Examples are superficial vascular cut down, and percutaneous biopsy.
STERILIZATION: The process whereby all viable microorganisms are eliminated or destroyed. The criterion of 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. Disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

Table 1 - RECOMMENDED HARD SURFACE DISINFECTANTS*

(e.g., table tops, equipment)

AGENT	EXAMPLES *	COMMENTS
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using.
Quaternary Ammonium	Roccal®, Quatricide® Tec-Surf II®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehydes (Cidex®, Cetylcide®, Cide Wipes®)	Rapidly disinfects surfaces.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

*Always follow manufacturer's instructions for dilution and expiration periods.

Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub (with soap) can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution (without soap). Alcohol, by itself, is not an adequate skin disinfectant. Since the evaporation of alcohol can induce hypothermia in small animals, avoid exposing excessively large areas.

* The use of common brand names as examples does not indicate a product endorsement.

Table 2 - SKIN DISINFECTANTS*

AGENT	EXAMPLES*	COMMENTS
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Cholorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

Table 3 - RECOMMENDED PROCEDURES FOR STERILIZING SURGICAL INSTRUMENTS*

AGENT	EXAMPLES**	COMMENTS
Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs 131°C for 3 min).
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue. *Only tips of instruments are sterilized with hot beads.*
Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time.
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcide®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
Hydrogen peroxide-acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.

*Always follow manufacturer's instructions for dilution, exposure times and expiration periods. ** The use of common brand names as examples does not indicate a product endorsement.

Table 4 - RECOMMENDED INSTRUMENT DISINFECTANTS*

AGENT	EXAMPLES**	COMMENTS
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using.
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.

Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

*Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

** The use of common brand names as examples does not indicate a product endorsement.

Table 5 - WOUND CLOSURE SELECTION

MATERIAL*	CHARACTERISTICS AND FREQUENT USES
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydiaxanone (PDS®) or, Polyglyconate (Maxon®)	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Polypropylene (Prolene®)	Nonabsorbable. Inert.
Nylon (Ethilon®)	Nonabsorbable. Inert. General skin closure.
Silk	Nonabsorbable. Restrict the use of silk to cardiovascular procedures or where silk's excellent handling properties are critical. Avoid for such purposes as routine skin closure since it may wick microorganisms into the wound, and cause tissue reactive.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®)	Skin glue. For non-tension bearing wounds. Note: use only products labeled for surgical use, super glue is not acceptable for surgery.

- Suture gauge selection: Use the smallest gauge suture material that will perform adequately.
- Cutting and reverse cutting needles: Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
- Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

* The use of common brand names as examples does not indicate a product endorsement.

Anesthesia

Staff veterinarians recommend the following anesthetics, routes and doses for use in commonly used rodents when the use of injectable agents is recommended. The veterinarians are available for consultation on these and other anesthetics and anesthetic protocols. All drugs, doses and routes of administration must be stated in the investigator's approved Animal Use Protocol (IACUP).

1. **Inhaled anesthetics**: Isoflurane delivered by mask or endotracheal tube via a precision

vaporizer is recommended for all species. Vaporizers are available for use in the Claverick and Coro procedure rooms. Contact ACF for information regarding vaporizer availability and training. For very brief procedures, (e.g., tail biopsies for genotyping), it may be acceptable to use isoflurane or other

Table 7 - Rodent Cocktail Solutions

inhalant anesthetics, without a precision vaporizer, in a "bell-jar" while precluding direct contact of animal skin with inhalant anesthetics. In all cases the anesthetic vapors must be adequately vented to prevent inadvertent exposure of personnel.

2. **Injectable anesthetics**: Injectable anesthetics are appropriate for many procedures. There is, however, a great deal of variation in depth and duration of anesthesia among rodent strains and individual animals.

Table 6 - Anesthesia

Anesthetic*	Mouse	Rat	Guinea Pig
Ketamine/ Medetomidine Cocktail[1]	75 mg kg^{-1} i/p 1 mg kg^{-1} i/p SQ or IP, may not produce surgical plane of anesthesia in some mice	75 mg kg^{-1} i/p 0.5 mg mg kg^{-1} i/p	40 mg kg^{-1} i/p 0.5 mg mg kg^{-1} i/p
Ketamine/ Xylazine Cocktail[2]		90 mg kg^{-1} i/p 90 mg kg^{-1} i/p	40 – 100 mg kg^{-1} 5 - 10 mg kg^{-1}
Pentobarbital[3]	40 – 85 mg kg^{-1} i/p	40 – 85 mg kg^{-1} i/p	28 mg kg^{-1} IP

[1] The 2 components can be mixed with water for injection (WFI) (USP water) – see table for Rodent Cocktail Solutions below.

[2] Xylazine is a potent respiratory depressant. Supplemental dosing, if necessary, should be done with ½ the original dose of ketamine alone.

[3] Consider replacing with inhalant or another injectable anesthetic.

	Mice	Rats	Guinea Pig
Ketamine mL (100 mg mL^{-1})		3.75 mL	3.75 mL
Xylazine (10 mg mL^{-1})		2.5 mL	2.5 mL
WFI		3.75 mL	3.75 mL

	Mice	Rats	Guinea Pig
Ketamine mL (100 mg mL^{-1})	0.5 mL	3.75 mL	2.0 mL
Medetomidine (1 mg mL^{-1})	0.5 mL	2.5 mL	2.5mL
WFI	4.0 mL	3.75 mL	5.5 mL

Total Volume		10.0 mL	10.0 mL		Total Volume	5.0 mL	10.0 mL	10.0 mL
Dosage		0.2 mL per 100gm	0.2 mL per 100gm		Dosage	0.1 mL per 10 gm	0.2 mL per 100gm	2.0mL per kg

Table 8 - Analgesics/Anti-inflammatory*

Agent	Mouse	Rat	Guinea Pig
Buprenorphine	0.05 – 0.1 mg kg^{-1} SQ Every 12 hours	0.05 mg kg^{-1} SQ Every 12 hours	0.05 mg kg^{-1} SQ Every 12 hours
Butorphanol	0.05 – 0.1 mg kg^{-1} SQ Every 4 hours	2 mg kg^{-1} SQ Every 4 hours	-
Aspirin	120 mg kg^{-1} PO	100 mg kg^{-1} PO	87 mg kg^{-1} PO
Carprofen	5 mg kg^{-1} SQ	5 mg kg^{-1} SQ	

* The IACUC will need to approve the exclusion of analgesics/anti-inflammatory agents for experimental surgery. If you feel that these agents are likely to interfere with the objectives of your research, include a scientific justification for their exclusion in your protocol.

5. Reference Sources:

- NRC (National Research Council), Institute of Laboratory Animal Resources, Commission on Life Sciences. 1996. Guide for the Care and Use of Laboratory Animals, Washington D.C., National Academy Press.
- The Animal Welfare Act, 7USC Sec 2131 et seq. Title 9, Code of Federal Regulations, parts 1, 2, & 3, January 1998.
- Laboratory Animal Anesthesia, A practical introduction for research workers and technicians., 2nd Edition, P.A. Flecknell (1996)

Back to top

5. Post-Op Animal Treatment Form

RHODE ISLAND HOSPITAL

RODENT POST OPERATIVE CARE FORM

Date & Time of surgery:_____

Investigator:_____ **Phone #:**_____ **Protocol #:**_____

Emergency Contact (name & phone #):_____

Species:_____ **Stock/ Strain:** _____ **Sex:**_____

Animal ID(s)/Cage ID(s):_____

Procedure: _____

Anesthetic agent(s): _____

Analgesic:_____ **Dose:**_____ **Frequency given:**_____ **# of Days:** _____

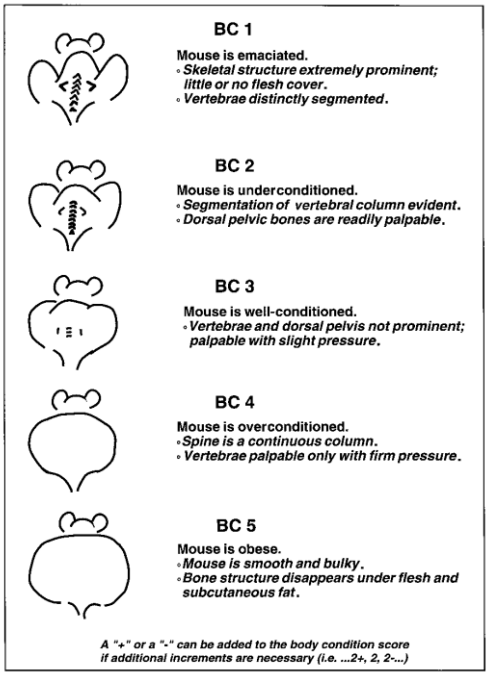
Other Medication(s): _____ **Dose:** _____ **Frequency/Days:** _____

Date	Time	Behavior, Appearance & Activity Assessment (Description)	Treatment/Medication	Initials

Some important post-operative/post-procedural parameters to consider:

- Assessment of Behavior, Appearance & Activity – are the animals bright, alert well-groomed and walking around the cage or are they quiet, scruffy and hunched in the corner? Do any post-op rodents have squinted eyes? Visual inspection of the cage before handling animals is important. Complete evaluation of animals in the hood can confirm your assessment.
- Body Condition Scores (BCS) – ideal scores fall within a range of 2+ to a 4- when palpation over the tail head.
- Weight – weight is a valuable tool when assessing the condition of your animals. Weight loss >15% from the pre-operative weight is considered significant and may be criteria for euthanasia.
- Fecal and Urine output – are there fecal pellets present in the cage?
- Incision site – is the surgical area clean and dry, is there discharge? Are all the sutures or wound clips present and intact?

Back to top



6. Post-Procedural Animal Treatment Form

RHODE ISLAND HOSPITAL

RODENT POST PROCEDURE CARE FORM

Date & Time of procedure:_____

Investigator:_____ **Phone #:**_____ **Protocol #:**_____

Emergency Contact (name & phone #):_____

Species:_____ **Stock/ Strain:** _____ **Sex:**_____

Animal ID(s)/Cage ID(s):_____

Procedure: _____

Anesthetic agent(s): _____

Analgesic:_____ **Dose:**_____ **Frequency given:**_____ **# of Days:** _____

Other Medication(s): _____ **Dose:** _____ **Frequency/Days:** _____

Date	Time	Behavior, Appearance & Activity Assessment (Description)	Treatment/Medication	Initials

Some important post-operative/post-procedural parameters to consider:

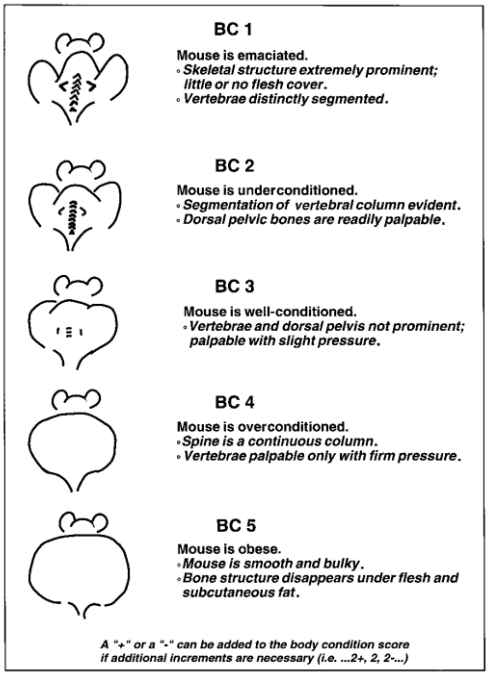
- Assessment of Behavior, Appearance & Activity – are the animals bright, alert well-groomed and walking around the cage or are they quiet, scruffy and hunched in the corner? Do any post-op rodents have squinted eyes? Visual inspection of the cage before handling animals is important. Complete evaluation of animals in the hood can confirm your assessment.

- Body Condition Scores (BCS) – ideal scores fall within a range of 2+ to a 4- when palpation over the tail head.

- Weight – weight is a valuable tool when assessing the condition of your animals. Weight loss >15% from the pre-operative weight is considered significant and may be criteria for euthanasia.

- Fecal and Urine output – are there fecal pellets present in the cage?

Back to top



7. Animal Health Program

SPECIES	DESCRIPTION*	PROCEDURE**
A. Dog	Class A. – Purpose bred from approved vendors. Vaccinated against: Canine Distemper Adenovirus Parainfluenza Parvovirus Leptospira Rabies	Physical examination and assessment when received at Rhode Island Hospital and Central Research Facilities (CRF) Review health report and vaccination history. Annual Vaccine
B. Cats	Closed Barrier Colony Vaccinated against: Faline Rhinotracheitis Calici Feline Luekema Panleukopenia Rabies	Physical examination and assessment when received at Rhode Island Hospital and Central Research Facilities (CRF) Review health report and vaccination history. Annual Vaccine
C. Swine	Closed Herd Validated free: Brucellosis Pseudorabies African Swine Fever	Observed for health problems upon receiving at CRF. House multiple animals together or in the same room, to the extent possible.

	Hog Colera Vaccinated: (6) strains Leptospirosis Sows vaccinated for bacterial and viral diseases Visually screened for umbilical and scrotal hernias and skeletal abnormalities Treated for parasites	Separate pigs from the approved vendors: EM Parsons & Sons, Inc., Marshall BioResources
D. Rabbit	1 Closed Colony 2 Semi-barrier	Physical examination of animals, tattooing for identification, or transporter chips.
* Vendor information supplied on request ** Newly arrived animals held separately for observations		

Rodent Health Monitoring

SPECIES	DESCRIPTION	PROCEDURE***
E. Mouse	Viral Antibody Free	Comprehensive diagnostic screen **** with full panel viral serology and Mycoplasma pulmonis, bacterial cultures from the respiratory and gastrointestinal tracts, examination for endo and ecto parasites, gross and histological examination. Serological testing***** for Sendi, MHV, and REO III. Examinationn for gross lestions.
F. Rats	Viral Antibody Free	Comprehensive diagnostic screen**** with full panel viral serology and Mycoplasma pulmonis, bacterial cultures from the respiratory and gastrointestinal tracts, examination

		for endo and ecto parasites, gross and histological examination. Serological testing***** for Sendi, Rat Corona, parvo and examinationn for gross lestions.
*** See quarantine period page 20 in RIH CAF Policies and Procedures Manual **** Five animals per room of VAF rodents including breeding colonies performed Oct/April. ***** Five animals per room of VAF rodents including breeding colonies performed Jan/July.		

Back to top

8. Cage Card Sample

LabTracks Sample Cage Cards

Animal ID:

Department:
Investigator:
Committee #:
Cost Center #:

Vendor:

Species:
DOB:
Sex:
Strain:

Entry Weight: _____

Date In:

Date Euth/ Initial:
Treatments/Comments:

_____
_____
_____

Back to top

9. Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols

Lifespan Central Research Facilities	Subject: **Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols**	Policy Number: **ORA-CAF Animal Care 18**
Issuing Department: Central Animal Facilities		Approved by: (CRF Director) (CRF Veterinarian)
Original Policy Date: 11/24/10 Latest Revision Date: 10/17/12 pab	**Page 1 of 5**	

1. **Purpose:**

Research projects that involve biohazardous agents (e.g. bacteria, viruses, parasites, and human derived materials) and chemical hazards (e.g. carcinogens, mutagens, teratogens, anti-neoplastic agents) must be classified by risk level, in accordance with the CDC *Biosafety in Microbiological and Biomedical Laboratories*. The IACUC, in conjunction with the Biohazard and Lab Safety Committee, will determine the hazard level for studies involving animals at the time of the protocol request. The nature of the hazard and Biosafety Level classification will be communicated to the Research and CAF staff through training meetings.

Rodents and other species involved in biohazard or chemical hazard research will be handled and housed in a manner to contain the hazard, and in an animal room separate from non-BSL-2 animals. The animal handling and husbandry procedures for these animals are contained in the following description.

2. **Requirements for ABSL-2 Containment:**

a. Biohazard/Hazard Signage

A hazard warning sign, incorporating the universal biohazard symbol or chemical symbol, as appropriate, will be posted on the access door to the animal holding room. The hazard warning sign will identify the agents in use, the biosafety level, and will list the names and telephone numbers of the Principal Investigator and other key laboratory personnel. It will also indicate the special requirements for entering and exiting the animal holding room. Contact information for the Safety Office and CAF personnel will be posted within the animal facility.

b. Access to Animal Housing Areas

Access to the animal housing facility is limited. Only those persons required for the experimental project or support purposes are authorized to enter the animal facility and the areas where animals that have been dosed with infectious materials and/or hazardous chemicals are housed or manipulated.

c. Animal Housing

Animals which have been treated with BSL-2 agents or hazardous chemicals will be kept in a room under negative pressure, and either housed in ventilated cage units that are under negative pressure, in a Modular Air Displacement (MAD) unit or in cages with microisolator filter tops on a static rack. The MAD units must be kept at a negative pressure to the room at all times. These animals will be grouped together on a rack or racks separate from unexposed animals.

1) **Biological Hazards**

- rDNA (recombinant DNA) - Cages of animals injected with Viral Vector Agents will not be touched for a period of 3 days (72 hours) after viral vector treatment unless required for emergent husbandry purposes (such as spilled water bottle or an escaped animal). In this event, Central Animal Facility (CAF) animal care staff will immediately contact the CAF Supervisor(s) and the Principal Investigator or their designee for direction in the care of the animal(s). CAF animal care staff will provide routine husbandry care for the life of the animal after the initial (3) day period.
- Human source tissues - Cages of immunodeficient animals (nude or SCID) injected with human source tissues or cell lines, will receive sterile cages and supplies, and will also be housed under ABSL-2 conditions for the life of the project.
- Live bacteria – Cages are considered BSL-2 for the life of the project.

2) **Chemical Hazards**

- Chemotherapy drugs and other hazardous chemicals - Cages of animals dosed with a hazardous chemical will be handled as recommended by the Biohazard Committee, as the number of days to wait before changing cages may differ. (In general, three days is adequate.)
- PCB agents do not excrete in urine or feces, but stay in the animal tissues. Cages may be disposed of as normal, non-contaminated work.

d. Animal Identification

Animals which have been treated with BSL-2 or chemical agents will be kept in cages with special card identification. The cage card must identify the hazard, the agent(s) dosed into the animals and the date of the procedure.

e. Work Environment

Work with animals exposed to BSL-2 agents, including the dosing and cage changes, must be done in a Class II biosafety cabinet or as specified by the Biohazards and Laboratory Safety Committee. All procedures must be carefully performed to minimize the creation of aerosols or splatter of infectious materials and waste.

Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.

f. Personal Protective Equipment

All personnel entering the animal BSL-2 holding room must wear double shoe covers, disposable gowns, appropriate respirator, typically N95 or PAPR (powered air purifying respirator), caps and gloves. This level of protection is sufficient if treated animals are to be handled in the holding room.
Personnel must also wear safety glasses if treated animals are to be manipulated. Eye protection, such as face shield or goggles, is required for anyone injecting hazardous agents into animals outside of a hood and is required for any procedure with a high potential for creating aerosols, such as necropsy of infected animals, harvesting of infected tissues or fluids and intranasal inoculations.

Before leaving the holding room, personnel will place disposable apparel items in a biohazardous or chemotherapy waste container, as appropriate. Gloves and PPE should be removed in a manner that prevents transfer of infectious materials.

g. Appropriate Disinfecting Agents and Methods

- Clidox® diluted at 1:18:1 will be provided as the disinfecting agent and to be used as a spray or to wipe on surfaces, or as a dip to decontaminate items. The contact time is a minimum of 5 minutes.
- An alternate agent would be 70% alcohol followed by a stabilized chlorine solution such as Dispatch®. Usually comes in a ready-to-use dilution, contact time 5 minutes.
- Bleach (Sodium Hypochlorite) at 1:10 dilution in cold water must be made fresh daily. Rapidly loses effectiveness in the presence of organic soil.
  The contact time is a minimum of 5 minutes.
- Care must be taken to observe the recommended expiration dates for the solutions.
- Unless specified, an alcohol-based hand sanitizer (e.g. Purell foam or liquid) is acceptable for decontamination of hands.
- Other disinfecting agents must be approved by CRF Management prior to use.

h. Work Surface Decontamination

Any work area(s) where animals treated with a BSL-2 or hazardous agent have been handled, either for project or cage changing purposes, must be decontaminated immediately after the termination of the activity. Note: These animals should always be the last ones worked with or changed by people entering the holding room. (See section g. above.) Paper or cloth products used for these applications will be placed in biohazardous waste.

i. Personal Hygiene

If other mice (with a different hazard) must be handled in the same Biohazard room after working with a group of treated animals, remove and dispose of gloves, cleanse hands or use hand sanitizer, then re-glove before proceeding to the other animals. Discarded protective items will be disposed of as biohazardous waste.

Before exiting the animal holding room, personnel who have handled any animal(s) dosed with a BSL-2 agent or hazardous chemical will use the proper method to cleanse their hands. Hand sanitizer may be used, unless specified. If hands are visibly soiled (e.g. blood, urine, feces), proceed directly to the nearest non-food area sink to wash

hands with the provided soap and water. Showering is recommended, but optional, after leaving the room.

j. Sharp Items

The use of syringes, needles, and sharps in the animal facility is limited to situations where there is no alternative to parenteral injection, blood collection, or aspiration of fluids from laboratory animals. Any sharp items used in the animal facility must be disposed of in a sharps container. Such containers will be readily available in the animal holding room. Only needle-locking syringes or disposable syringe-needle units are to be used for these purposes. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal in the sharps container. Chemotherapy needles and syringes are placed in a yellow sharps bin.

k. Spills

Any spill and/or accident which results in overt exposure to biohazardous or hazardous material will be immediately reported to the appropriate Safety Officer, key laboratory personnel and Central Research Facilities/Central Animal Facilities management. Contact information for the Safety Officer and CRF/CAF management is posted within the animal facility. The Safety Officer will report the incident to the appropriate safety committee(s) at the next scheduled meeting.

l. Transporting Rodents and Tissues Treated with a BSL-2 Agent

Rodents which have been treated with a BSL-2 or hazardous chemical agent must be moved within the animal facility or between the animal facility and the laboratory in a clean shoebox cage fitted with a Microisolator filter top. The shoebox cage lid must be secured either by wrapping a large elastic band around the container or using a binder clip to secure the filter top; wiped with appropriate disinfectant and then placed inside a secondary transport container (tote box or enclosed cart). The container must be dedicated for the transport of biohazardous animals, be labeled as such, and approved by CAF Management. BSL- 2 precautions must be observed wherever filter tops are removed from cages or animals are handled for any purpose.

Biological material removed from animals which have been treated with a BSL-2 agent will be transported to the investigator's laboratory for analysis in a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility.

m. Cage Changing (Shoe Box Type)

Surface decontamination procedures (see above) will be carried out before and after rodent cages are changed. For BSL-2 animals, all dirty caging will be securely wrapped or bagged in an autoclavable plastic bag to prevent exposure during transport to the cage processing area.

- The cages with bedding will be autoclaved prior to being dumped. Stainless steel wire cage lids and microisolator cage tops or vent rack lids will be changed every two weeks and autoclaved before washing. Water bottles are autoclaved prior to washing.

- If cages cannot be autoclaved, such as with hazardous chemicals or chemotherapy agents, bedding may be emptied into doubled red plastic biohazard bags in a dump station in the cage wash area or in a Class II biosafety cabinet by personnel wearing gowns, gloves and appropriate respiratory protection. Cages will be wiped clean with a disposable wipe moistened with Clidox® 1:18:1 solution to remove debris, then thoroughly wetted with Clidox® solution for at least five minutes. An alternate method would be 70% alcohol wipe down, followed by a spray or wipe with a stabilized chlorine solution such as Dispatch® and leave the items to dry, before being washed mechanically. Wire lids and filter tops will be treated in a similar manner with Clidox® and mechanical washing.
- If disposable cages are used, bag the cages in red bags, place in biohazard boxes and mark boxes for incineration.
- Waste Clidox® solution is to be washed down the drain with excess water.

n. Disposal of Animal Carcasses

The carcasses of mice treated with a BSL-2 agent or hazardous chemical are to be double bagged in plastic, with the outer bag being a biohazard bag. The outer bag will be sprayed or wiped with appropriate disinfectant prior to leaving the room. Bags are stored in the animal facility freezer until removed and transported for incineration. For rDNA projects, investigators are responsible for maintaining a permanent record of animal use and disposition for each animal or group of animals (NIH Recombinant DNA Guidelines, Appendix Q-1-B-2)

o. Waste

Trash containers in the BSL-2 room will be marked with a biohazard sticker and lined with a red bag. Containers for chemotherapy waste are puncture proof yellow plastic and identified as chemo waste only. All waste, including disposable apparel, will leave the room as biohazardous or chemotherapy waste.

3. Records, Forms and Reports:

Biohazard or hazardous chemical sign on door and on cage cards

4. Reference Sources:

- Centers for Disease Control and Prevention; The National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC. 2009. http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- Department of Health and Human Services, The National Institutes of Health. NIH Guidelines for Research Involving Recombinant DNA Molecules http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

Back to top

10. Cadaver and/or Animal Parts Form

Cadaver and/or Animal Parts Form

Instructions: Completion of this form is only required for those cadavers or animal parts that are obtained from sources outside of Rhode Island Hospital/Lifespan Corporation. Such sources may include but are not limited to slaughterhouses; other academic, private-industry or government research facilities or commercial vendors. Please refer to the Rhode Island Hospital IACUC Policy on Review of Cadavers or Animal Parts used in Research for additional information.

Today's Date:_____

Name:_____

Address:_____

_____

Phone Number: _____ E-mail Address: _____

Species: _____ Tissue(s) required: _____

Date Needed: _____

Source of cadaver/parts: _____

Is there a known or potential hazard/infectious diseases associated with this tissue? Yes No, If yes, please specify

Biohazard

Recombinant DNA

Chemical Hazard

Radioactive Hazard

Additional review by EH&S may be required if hazardous tissues are to be utilized for research purposes.

Intended use:

Disposal method:

For CRF Veterinary Staff Use Only

Reviewer: Approval Date:

EH&S Reviewer (if applicable):

Back to top

11.Tumor Monitoring Form

TUMOR MONITORING							
Start Date:	Protocol number:	PI name and email:	Lab contact name and email:	Phone:			
Frequency of Monitoring (per ACUP)	Experimental End Points (per ACUP)						
Observation Codes: P= Tumors have not reached protocol specific end point, U= Ulceration, D= Found dead, E= Euthanized (indicate number of animals with observation codes U, D, or E)							
Date:	Observation code:	Cage number:	Initials:	Date:	Observation code:	Cage number:	Initials:

Back to top

12. Notice of Intent to Use Avian Embryos Form

Notice of Intent to Use Avian Embryos

Project Title:
Principal Investigator:
Department:
Email:
Phone:

Avian Embryo Use Summary

1. Avian Species to be Used.
(Specify all species, typical incubation for each, and incubation at planned use)

Species	Length of Normal Incubation	Embryo Age(s) at Planned Use
☐ Chicken	21 days	
Other - Specify		

(Note: Add or delete rows as necessary)

2. Building and room number where avian embryo use will occur

3. Method of euthanasia of embryos < 50% incubation (≤10 days for chickens)
☐ Not applicable. Embryos will be used after 50% incubation

4. Method of euthanasia of embryos > 50% incubation (≥11 days for chickens)
(Specify for all species, in the event planned use is delayed for some reason)

5. Procedure for euthanasia of inadvertently hatched chicks
(See AVMA Guidelines for the Euthanasia of Animals: 2013 and/or consult veterinarians)

Investigator Assurance

I have read the Lifespan IACUC "Policy for Use of Avian Embryos" and agree to abide by it. (See CRF Policy & Procedure Manual, Section VII.M)

Signature _____ Date _____

Back to top