MARSHALL UNIVERSITY
JOAN C. EDWARDS SCHOOL OF MEDICINE
ANIMAL RESOURCE FACILITY
POLICY AND PROCEDURES MANUAL

Byrd Biotechnology Science Center

Director: Billy W. Howard, DVM

Hours: 7:30 a.m. to 3:30 p.m. Monday through Friday
Limited hours on Saturday, Sunday and Holidays

2016

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SOP INDEX

I. Introduction

A. The primary objective of the Association for Assessment and Accreditation, (AAALAC) International accredited Animal Resource Facility (ARF) is to provide professional animal support services to the faculty, staff and students at Marshall University.

B. The use of animals for teaching and research is a fundamental part of biology and medicine. Suitable animals are required for investigative and teaching purposes. Proper care and management of these animals is both a scientific necessity and a legal requirement.

C. Animals are sentient beings. Their use is a privilege granted to the scientific community by the public and its policy-making institutions. Along with this privilege goes the ethical responsibility for their humane care and proper use. The University, the individual investigator and each member of his staff must share in this partnership of responsibility.

D. This guide describes the organization of the ARF, the policies which govern its operations, and its basic rules and regulations. It should be reviewed by all personnel who are involved with the use of animals on this campus. Policies and procedures will be subject to change with changing conditions and new information.

II. Organization

A. The ARF is a centralized service organization responsible for the procurement of animals, cages, feed, supplies and related items. It is responsible for the daily husbandry and care of experimental animals, and their general health.

B. The Director of the ARF is a veterinarian, whose major responsibility is the efficient functioning of the unit. His professional qualifications and interests lie in the specialty of Laboratory Animal Medicine, and he serves the entire campus in all matters of the Veterinary Sciences. His duties are diversified, including administrative, clinical, teaching, consultative, and research activities.

C. The ARF Director is responsible for complying with and implementing all aspects of the "Laboratory Animal Welfare Act" (P.L. 89-544 and amendments). He will also comply with the following guidelines.

1. Guide for the Care and Use of Laboratory Animals. 8th edition, NRC

2. "Grants Administration Manual." DHHS

3. AVMA Guidelines for the Euthanasia of Animals.

4. Applicable local and state codes.

5. Standards established by other granting agencies, University Policy and appropriate Professional Societies.

D. The Institutional Animal Care and Use Committee is composed of members qualified through experience and expertise to oversee the institution's animal care programs. The principal charges to this committee are:

1. To review all research protocols in which laboratory animals are used to ensure compliance with the Animal Welfare Act and other local, state and federal rules, regulations and guidelines for humane care and use of animals.

2. To review the institution's program for humane care and use of animals once every six months. A report of the review is to be submitted to the Institutional Official.

3. To inspect at least once every six months all of the institution's animal facilities, including satellite facilities and animal study areas. A report of this inspection is to be submitted to the Institutional Official.

4. To make recommendations to the Institutional Official regarding any aspect of the research facility's animal program, facilities, or personnel training.

5. To serve as advisory adjudicating body to the Institutional Official or his designee on disagreements on the animal resources operations and animal welfare.

6. To handle other matters relating to the operation of the animal resource facility and animal welfare and usage as requested by the Institutional Official or his designee.

III. **Principles and Guidelines**

A. The ARF operates as a centralized laboratory facility in accordance with the operating concepts and procedures in the NRC publication, Guide for the Care and Use of Laboratory Animals, 8th edition.

B. The following principles shall be applied for the utilization and care of vertebrate animals used in testing, research and training.

1. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seq.) and other applicable Federal laws, guidelines and policies.

2. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of the society.

3. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.

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

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

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

7. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

8. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

9. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle 2, by an appropriate review group such as the Institutional Animal Care and Use Committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

IV. **Standard Procedure**

A. Animal Acquisition

1. An "Application for the Care and Use of Laboratory Animals" must be completed by the principal investigator and reviewed by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of a teaching or research project. The Application for the Care and Use of Laboratory Animals is available on the website www.IRBNet.org or at the ARF Website, http://musom.marshall.edu/arf/. The application and review are a part of our commitment to comply with the amended Animal Welfare Act. The Public Health Service policy of Humane Care and Use of Laboratory Animals and the USDA Animal Welfare Act Regulations to review protocols involving animals. The completed application should be submitted to the IACUC through the www.IRBNet.org website for administrative review, and placed on the agenda of the next IACUC meeting. The application is then reviewed by the IACUC to determine:

    a. Rationale for involving, and the appropriateness of the species and numbers of animals to be used.

    b. Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals.

    c. The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals.

    d. Written assurance has been provided that the activities do not unnecessarily duplicate previous experiments.

    e. Procedures that cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics, or anesthetics, unless withholding such agents is justified, in writing, for scientific reasons.

    f. Animals are humanely euthanized by AVMA Guidelines for the Euthanasia of Animals.

    There are special sections in the application for surgery, hazardous agents, and prolonged restraint. These must be completed if they are pertinent to the protocol.

Qualifications and experience of the personnel conducting the procedures are included and evaluated by the IACUC.

Applications and amendments may be submitted and considered outside the scheduled meeting; however, every committee member must review the protocol or amendment. There are no expedited reviews. Final review and approval of the full IACUC must be complete and the Principal Investigator notified before the work can begin. All animal use, regardless of funding, undergoes the same review process. All approved studies undergo annual review.

Upon approval of the IACUC, the investigator must complete an "Animal Request Form" in full before animals will be ordered. These forms are available on the website www.IRBNet.org. The ARF will be able to supply cages for the majority of anticipated caging needs. If the need arises for housing that cannot be supplied by the ARF, some arrangements must be made to assure that proper facilities are provided before the animals are ordered.

2. An Animal Request Form is used to order animals through the ARF. This form, properly filled out and approved by the fiscally responsible person, must be received in the office of the ARF Director in sufficient time to allow for acquiring, processing, and delivery of the animals ordered. The ARF will order the animals and the investigator's department will be notified by telephone that the animals have been received and the room number where the animals will be housed after quarantine. All animals are ordered by ARF. This is so preparation can be made for incoming animals. Requests for the most commonly used animals need to be in the ARF office by 12:00 pm on Wednesdays to ensure shipment the following week.

3. Make sure that you order what you want, and that sufficient time is allowed to acquire, process, and receive the order to your specification. With some animals, a minimum time of less than four (4) weeks between placing of orders and desired delivery may prove disappointing. Acquisition of transgenic or mutant animals, may require longer.

4. All new shipments of animals will be held in quarantine for various lengths of time. This is to establish their health status and allow for acclimation before they are released for investigator use and introduced to our resident animal population.

B. Animal Housing

1. Animal housing and support functions provided by the ARF are directed toward serving the interests of its users. Effective space utilization is generally best accomplished by housing identical species for numerous investigators in the same room, as opposed to assigning each investigator or department an individual room. When research protocol or scientific

judgment dictates that the above system of animal housing is unacceptable, exceptions can be made. In such cases, the ARF Director, in consultation with the investigator, can assign ARF room (if available) to the investigator, or else the investigator can provide his own areas for animal housing if this is physically acceptable. In both cases, if the ARF does not provide total animal care, the individual must provide in a humane, legally prescribed manner the complete environmental care and sanitation needs of the involved animals as outline by the ARF Director. The only exception to this would be documented evidence that these procedures will interfere with research objectives.

2. Minimum standards pertaining to space required for each animal have been established. When animals are found in cages which are too small or they have outgrown cage sizes considered adequate when they arrived, they will be moved into adequate sized units. Since this could be disruptive to experiments, it is advisable that all investigators doing long-term work maintain a liaison with the ARF personnel to assure that adequate cage requirements are met.

3. Animals will not be kept in research laboratories overnight unless protocol dictates and prior approval from the IACUC has been granted.

4. Animals shall be transported from animal rooms in approved carrying cases or cages.

5. The use of animal rooms for purposes other than housing animals is discouraged because of possible hazardous effects on the other animals. There is no provision for investigators' records or equipment to be kept in the animal rooms. For investigator convenience, any qualified investigator may use the procedure room for animal manipulations. It is requested that the use of the room be scheduled through the ARF office. If scheduled, the room will be available to you at the scheduled time. This room will be clean when you use it, and is expected to be clean before you leave it.

6. Casual visitors and pets are not allowed in the animal facilities.

7. Smoking and eating will not be permitted in any of the animal rooms.

C. Animal Care

1. The total maintenance program of all animals housed in the ARF will be conducted by trained ARF personnel. This program includes daily cage cleaning, feeding and watering along with the routine cage washing, treatment for naturally occurring diseases and other needs that may arise in regard to good animal husbandry practices. The ARF daily maintenance includes all routine care by personnel. Included are feeding of standard diets, ad

lib; watering daily, ad lib; provision of bedding; routine cleaning and sanitizing of cages, and routine cleaning of the rooms.

2. Special diets provided by investigators can be fed by ARF personnel *ad lib*, or in prepackaged units or in easily measured quantities. This applies to situations where very little, if any, record of consumption or spillage is required. Potentially hazardous agents will be fed by ARF personnel only after approved by the IACUC.

3. Any animal that is sick, diseased, or injured must be provided with veterinary care or humanely destroyed. When therapy is indicated, the investigator is notified before recommended treatment is begun. If it is impossible to contact the investigator within a reasonable period of time, critically ill or injured animals will be euthanized. The only exceptions are those disabilities which are an integral part of an appropriately approved project. These procedures must be carefully supervised by the principal investigator or other qualified scientist. The scientist in charge of the experiment must be prepared to terminate it whenever its continuation may result in unnecessary injury or suffering to the animal.

D. Animal Disposition

1. After completion of an animal experimentation project, investigators will be responsible for the disposition of the animals. The ARF Director will be available for consultation on the accepted methods of euthanasia. There is no place in the ARF where ether can be used safely. If animals are euthanized with ether, under no circumstances should the carcasses be placed in the cold room before the odor of ether has been dissipated in a hood. No animal should be discarded until death is certain.

2. After investigators complete their animal experimentation projects, they should place all animal carcasses or animal parts that are no longer needed in a plastic bag and deposit them in the cold room. ARF personnel will be responsible for the disposition of the animal carcasses.

3. In the event an experimental animal should die, ARF personnel will place it in the cold room (116A) and an "Animal Death Report" will be completed. The responsible investigator will be notified as soon as possible. The animal carcass will be held in the cold room for 24 hours and then disposed of. If an investigator desires to have his animal held for a longer period of time, he should contact the ARF Director.

4. Research animals are considered state property and none may be retained or given as pets.

E. Special ARF Services

1. Veterinary consultation is encouraged and will be available for an investigator who desires this service for University related teaching or research needs. Consideration might be given to such things as:

    a. Suitable animals for the experimental design.

    b. Sources of animals and equipment.

    c. Necropsy results.

    d. Per diem charges.

    e. Unusual environmental requirements.

    f. Special diets and care.

    g. Pre- and post-operative care.

    h. Special nursing care.

    i. Analgesics.

    j. Other professional, technical, and ancillary services.

2. ARF personnel will be available for assistance in handling and restraint of some of the larger and more difficult animals for short periods of time.

F. Billing

1. The ARF will prepare monthly billings for investigators who have utilized the services. The statement will be itemized to indicate the number of board days per species, animal purchases, and other charges for specific items that have been requested. This statement will be forwarded to the Investigator's Department where the amount of each investigator's charges is encumbered by the Department Secretary on the Banner System.

2. The School of Medicine is absorbing many of the costs inherent to the operation of the ARF, such as transportation from the airport to the school, maintenance of the facility, disposal of animals, etc.

G. Animal Hazards and Zoonotic Diseases

1. The hazards associated with handling animals can be loosely placed in three categories. First, physical injuries occur from bites or scratches (rodents, rabbits,). The key to prevention of these types of injuries is proper training of research personnel by the animal care staff or other qualified individuals. Second, there are serious allergic hazards associated with breathing or contacting allergens such as animal dander or urine. The safest policy is to prevent exposure by always wearing protective clothing (such as face masks, gloves, and a lab jacket) when handling animals. Third, the possibility of zoonotic diseases must always be considered. Zoonotic diseases are those that can be transmitted from animals to humans. Although zoonotic diseases are not common in modern facilities, the prevention, detection, and eradication of zoonotic diseases from the animal facility is a primary concern of the entire animal care staff. Remember that zoonotic diseases can frequently be transmitted by tissues as well as the living animals.

   Although humans usually are not susceptible to infectious diseases suffered by animals, there are some important exceptions. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if any, sign of illness. A pathogen in the normal flora of a healthy animal may cause a serious disorder in a person exposed to it because the animal has developed "resistance" to these microorganisms, whereas humans with no previous exposure to the agent lack this protective immunity. Therefore, one should always be aware of possible consequences when working with each species of animal, and take precautions to minimize the risk of infection. In the event that you do become ill with a fever or some other sign of infection, it is important to let the physician caring for you know that you work with animals.

   Some of the specific diseases and the animals associated with those disorders are described below.

   There are some common sense steps that can be taken to lessen the risk of infection in general. These include no eating, drinking, or applying cosmetics or contact lenses around animals or animal care areas, wearing gloves when handling animals or their tissues, taking care not to accidentally rub your face with contaminated hands or gloves, and hand washing after each animal contact. Research personnel can protect themselves against self-inoculation by wearing gloves, substituting manually operated pipettes for needles and syringes, taking enough time to give injections properly, anesthetizing animals prior to inoculation with infectious agents, and using a two-person team to inoculate animals. Do not recap used needles! Instead, discard them promptly in a biohazard "sharps" container. For procedures such as necropsies, bedding changes, and tissue and fluid sampling, physical containment devices such as biological safety cabinets, full-face respirators or other personal safety gear should be used as indicated.

The scope of possible zoonotic infections is quite large, and only a few examples will be described here. However, all personnel should be aware that laboratory animals are sources of potent allergens to sensitized persons. Further details are available from the Division of Occupational and Environmental Health.

a. If you work with rodents (e.g., Mice, Rats)

1) Contact with rodents requires precautions against such diseases as tapeworm infection, salmonellosis, and "ringworm" fungal skin infections. Additional concerns for investigators using some rodents are leptospirosis and bubonic plague. Attention should also be paid to the possibility of allergic reactions.

To protect against these agents, care should be taken to limit exposure to soiled bedding containing feces (salmonellosis, tapeworms) and urine (leptospirosis). Gloves and masks not only limit exposure to soiled bedding, but also help prevent transmission of diseases such as ringworm and fur mites when rodents are handled.

There are infectious agents that can be transmitted to humans through rodent bites, but the incidence of these agents in modern rodent colonies is virtually non-existent.

b. Occupational Health and Safety Program

The occupational health program is voluntary but highly encouraged for all members of Marshall University who work in laboratory animal facilities or have substantial animal contact. This includes animal resource personnel, research technicians, research investigators, faculty, and staff. The program consists of the following, if deemed necessary by the Occupational Health Physician:

1. Completion of Occupational Health Questionnaire
2. Review of the health questionnaire and risk assessment performed by the Occupational Health Physician who will determine which of the following procedures will be performed on the individual.
3. CBC with differential (annually)
4. Urinalysis (annually)
5. TB testing (annually), if test result is positive, employee will be given chest x-ray
6. Tetanus immunization

If deemed necessary by the Occupational Health Physician, the following vaccines shall be offered and if declined, a declination form will be signed and placed in employee personnel folder. If employee chooses at a later date to receive the vaccine, employee may do so.

Rabies prophylactic series
Hepatitis B vaccine series

These procedures shall be offered at no cost to the employee and shall be conducted under the direction of a licensed health care professional.

Medical records shall be maintained in employee's personnel folder and shall be provided upon written request for copying to the subject employee or to anyone having written consent of the subject employee.

Department chairpersons/managers are responsible for disseminating this program to their respective department personnel. These same people are responsible for enrolling their personnel in the program and providing the above tests, procedures, and vaccines to the employee.

H. Procedures for reporting exposure and potential hazards.

Personnel will notify their supervisor of potential or known exposures and of suspected health hazards and illnesses. The supervisor will record this exposure or illness and contact the emergency room for instructions. If the employee's supervisor is not available, he will contact the IACUC Chairman or the Veterinarian for instructions.

Suspected health hazards will be reported to the supervisor, IACUC Chairperson, or the Veterinarian for corrections.

I.

ANESTHESIA AND ANALGESIA IN LABORATORY ANIMALS AT MARSHALL UNIVERSITY

Mouse Formulary

INHALATION ANESTHETICS:

Drug Name	Dose (mg/kg) & Route	Frequency	Notes
Isoflurane	**1-3% inhalant to effect; usually 5% for induction**	**Whenever general anesthesia is required;**	**Use precision vaporizer. Deliver with 1.0-1.5% oxygen. Survival surgery requires pre-emptive analgesia.**
Carbon Dioxide	**From a compressed gas cylinder, 70% v/v to effect and for 1 minute past apparent death**	**Once at time of euthanasia**	**Must be followed by a secondary means of euthanasia, such as rapid cervical dislocation.**

INJECTABLE ANESTHETICS:

Drug Name	Dose (mg/kg) & Route	Frequency	Notes
Recommended: **Ketamine (K)+** **Xylazine (X) +** **Acepromazine (A)** **(in same syringe)**	**75 (K) +** **7.5 (X) +** **1.5 (A)** **IP**	**As needed** **Volume: 0.1 ml/10 grams mouse body weight**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**
Ketamine (K)+ **Xylazine (X) +** **Acepromazine (A)** **(in same syringe)**	**70-100 (K) +** **10-20 (X) +** **2-3 (A)** **IP**	**As needed**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**

Ketamine (K) + Medetomidine (M) (in same syringe)	**50-75 (K) + 0.5-1 (M) IP**	**As needed**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**
Ketamine (K) + Xylazine (X) (in same syringe)	**80-100 (K) + 5-10 (X) IP**	**0.1 ml/10 grams body weight per mouse**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**

REVERSAL AGENTS:

Drug	Dose (mg/kg) & route	Frequency	Notes
Atipamezole	**0.1-1.0 SC or IP**	**Any time medetomidine or xylazine has been used.**	**Atipamezole is dosed at the same volume as medetomidine, but they are manufactured at different concentrations.**
Yohimbine	**1.0-2.0 SC or IP**	**For the reversal of xylazine**	

OTHER INJECTABLE ANESTHETIC AGENTS:

Drug	Dose (mg/kg) & route	Frequency	Notes
Sodium pentobarbital	**40-50 IP**	**Recommended for terminal/acute procedures only; booster doses as needed.**	**Consider supplemental analgesia (opioid or NSAID) for invasive procedures.**

ANALGESIC: OPIOID

Drug	Dose(mg/kg) & route	Frequency	Notes
Buprenorphine	**0.05-0.1 SC or IP**	**Used pre-operatively for preemptive analgesia and post-operatively every 4-12 hours**	**When used as a sole analgesic agent, this is the typical regimen: once at time of procedure, second dose 4-6 hours later; additional doses every 8-12 hours as needed. Consider multi-modal analgesia with an NSAID and local analgesic agent.**

ANALGESIC: NSAID (Non-steroidal anti-inflammatory analgesia) (Note: prolonged use may cause renal or gastro-intestinal problems)

Drug	Dose (mg/kg) & route	Frequency	Notes
Recommended: Carprofen	**5-10 SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours**	**Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine**
Ketoprofen	**2-5 SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours**	**Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine**
Ketorolac	**5-7.5 oral or SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours**	**Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine**
Flunixin meglumine	**2 SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours**	**Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine**

ANALGESIC: LOCAL ANESTHETIC/ANALGESIC (lidocaine and bupivicaine may be combined in one syringe for rapid onset and long duration of analgesia)

Drug	Dose (mg/kg) & route	Frequency	Notes
Lidocaine hydrochloride	**Dilute to 0.5%, and do not exceed 7 mg/kg total dose. SC or Intra-incisional**	**For use locally before making a surgical incision or before the skin is sutured.**	**Faster onset than bupivicaine but < 1 hour of action**
Bupivicaine	**Dilute to 0.25% and do not exceed 8 mg/kg total dose. SC or Intraincisional**	**For use locally before making a surgical incision or before the skin is sutured.**	**Slower onset than lidocaine but > 4-8 hours of action**

REFERENCES:

Lumb and Jones' Veterinary Anesthesia, 3rd edition; Thurmon JC, Tranquilli WJ, Benson GJ; Lippincott, Williams, & Wilkins Publishing; Section VII, Chapter 21, p.686-735.

Formulary for Laboratory Animals, 3rd edition; Hawk CT, Leary SL, Morris, TH; Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014.

Plumb's Veterinary Drug Handbook, 5th edition; Plumb DC, Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014.

Rat Formulary

INHALATION ANESTHETICS:

Drug Name	Dose (mg/kg) & Route	Frequency	Notes
Isoflurane	**1-3% inhalant to effect; usually 5% for induction**	**Whenever general anesthesia is required;**	**Use precision vaporizer. Deliver with 1.0-1.5% oxygen. Survival surgery requires pre-emptive analgesia.**
Carbon Dioxide	**From a compressed gas cylinder, 70% v/v to effect and for 1 minute past apparent death**	**Once at time of euthanasia**	**Must be followed by a secondary means of euthanasia, such as rapid cervical dislocation.**

INJECTABLE ANESTHETICS:

Drug Name	Dose (mg/kg) & Route	Frequency	Notes
Recommended: **Ketamine (K) +** **Xylazine (X)** **(in same syringe)**	**80-100 (K) +** **5-10 (X)** **IP**	**0.2 mL/100 grams body weight as needed**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**
Ketamine (K)+ **Xylazine (X) +** **Acepromazine (A)** **(in same syringe)**	**70-100 (K) +** **10-20 (X) +** **2-3 (A)** **IP**	**0.1 mL/100 grams body weight as needed**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**
Ketamine (K) + **Medetomidine (M)** **(in same syringe)**	**75-100 (K) +** **0.5-1 (M)** **IP**	**As needed**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**
Ketamine (K) + **Xylazine (X)** **(in same syringe)**	**80-100 (K) +** **5-10 (X)** **IP**	**0.2 ml/100 grams per rat**	**May not produce surgical plane of anesthesia; if redosing, use ketamine alone. May be partially reversed with atipamezole or yohimbine.**

REVERSAL AGENTS:

Drug	Dose (mg/kg) & route	Frequency	Notes
Atipamezole	**0.1-1.0 SC or IP**	**Any time medetomidine or xylazine has been used.**	**Atipamezole is dosed at the same volume as medetomidine, but they are manufactured at different concentrations.**
Yohimbine	**1.0-2.0 SC or IP**	**For the reversal of xylazine**	

OTHER INJECTABLE ANESTHETIC AGENTS:

Drug	Dose (mg/kg) & route	Frequency	Notes
Sodium pentobarbital	**40-50 IP**	**Recommended for terminal/acute procedures only; booster doses as needed.**	**Consider supplemental analgesia (opioid or NSAID) for invasive procedures.**

ANALGESIC: OPIOID

Drug	Dose(mg/kg) & route	Frequency	Notes
Buprenorphine	**0.01-0.05 SC or IP**	**Used pre-operatively for preemptive analgesia and post-operatively every 4-12 hours**	**When used as a sole analgesic agent, this is the typical regimen: once at time of procedure, second dose 4-6 hours later; additional doses every 8-12 hours as needed. Consider multi-modal analgesia with an NSAID and local analgesic agent.**

ANALGESIC: NSAID (Non-steroidal anti-inflammatory analgesia) (Note: prolonged use may cause renal or gastro-intestinal problems)

Drug	Dose (mg/kg) & route	Frequency	Notes
Recommended: Carprofen	4-5 SC	Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours	Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine
Recommended: Ketoprofen	2-5 SC	Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours	Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine
Ketorolac	5-7.5 oral or SC	Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours	Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine
Flunixin meglumine	2 SC	Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hours	Depending upon procedure may be used as sole analgesic agent or as multi-modal analgesia with buprenorphine

ANALGESIC: LOCAL ANESTHETIC/ANALGESIC (lidocaine and bupivicaine may be combined in one syringe for rapid onset and long duration of analgesia)

Drug	Dose (mg/kg) & route	Frequency	Notes
Lidocaine hydrochloride	Dilute to 0.5%, and do not exceed 7 mg/kg total dose. SC or Intra-incisional	For use locally before making a surgical incision or before the skin is sutured.	Faster onset than bupivicaine but < 1 hour of action
Bupivicaine	Dilute to 0.25% and do not exceed 8 mg/kg total dose. SC or Intraincisional	For use locally before making a surgical incision or before the skin is sutured.	Slower onset than lidocaine but > 4-8 hours of action

REFERENCES:

Lumb and Jones' Veterinary Anesthesia, 3rd edition; Thurmon JC, Tranquilli WJ, Benson GJ; Lippincott, Williams, & Wilkins Publishing; Section VII, Chapter 21, p.686-735.

Formulary for Laboratory Animals, 3rd edition; Hawk CT, Leary SL, Morris, TH; Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014.

Plumb's Veterinary Drug Handbook, 5th edition; Plumb DC, Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014.

RABBIT FORMULARY

Note that all of these doses are approximations and must be titrated to the animal's strain, age, sex and individual responses. Significant departures from these doses should be discussed with a veterinarian. Doses will also vary depending on what other drugs are being administered concurrently.

All doses are listed as milligrams per kilogram (mg/kg) unless otherwise noted.

DRUG NAME	DOSE (MG/KG) & ROUTE	FREQUENCY	NOTES
Inhalation anesthetics			
Recommended: Isoflurane	**1-3% inhalant to effect (up to 5% for induction). Up to 8% for Sevoflurane**	**Whenever general anesthesia is required**	**Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer. Mask or chamber induction without injected pre-medication may result in breath-holding and injury.**
Ketamine combinations			
Recommended: Ketamine-Xylazine	**35 – 50 + 5-10 IM or SC (in same syringe or with xylazine administered 10-20 minutes in advance)**	**As needed**	**May not produce surgical-plane anesthesia for major procedures. If redosing, use ketamine alone. May be partially reversed with Atipamezole or Yohimbine. Note that IM Ketamine combinations often sting upon injection.**

Ketamine alone	**20 – 60 IM or SC**	**As needed**	**Deep sedation, but not surgical anesthesia. Not often used alone. Note that IM Ketamine combinations often sting upon injection.**
Ketamine-Medetomidine	**35 - 50 + ~ 0.5 IM or SC (in same syringe, or with medetomidine administered 10-20 minutes in advance)**	**As needed**	**May not produce surgical-plane anesthesia for major procedures. If redosing, use ketamine alone. May be partially reversed with Atipamezole. Note that IM Ketamine combinations often sting upon injection.**
Ketamine-Xylazine-Acepromazine	**35-40 + 3 - 5 + 0.75 – 1.0 IM or SC (in same syringe)**	**As needed**	**May not produce surgical-plane anesthesia for major procedures. If redosing, use ketamine alone. May be partially reversed with Atipamezole or Yohimbine. Note that IM Ketamine combinations often sting upon injection.**
Ketamine-Midazolam	**35 - 50 + ~ 2 IM or SC (in same syringe)**	**As needed**	**May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint. Note that IM Ketamine combinations often sting upon injection.**
Reversal agents			
Atipamezole	**0.1 - 1.0 subcutaneous or IP**	**Any time medetomidine or xylazine has been used**	**More specific for medetomidine than for xylazine (as a general rule, Atipamezole is dosed at the same *volume* as Medetomidine, though they are manufactured at different concentrations)**
Yohimbine	**~ 0.2 IV or SC**	**For reversal of xylazine effects**	
Other injectable anesthetics			
Sodium pentobarbital (Nembutal)	**20 - 60 IV**	**Recommended for terminal/acute procedures only, with booster doses as needed**	**Consider supplemental analgesia (opioid or NSAID) for invasive procedures. Apnea is common at anesthetic doses.**

Propofol	**12-26 IV**	**As needed**	**Only useful IV, so therefore limited usefulness. Respiratory depression upon induction is possible.**
Opioid analgesia			
Recommended: Buprenorphine	**0.05 - 0.1 SC or IP**	**Used pre-operatively for preemptive analgesia and post-operatively every 4-12hrs**	**When used as sole analgesic, typical regimen is: once at time of procedure, second dose will be administered 4-6 hours later. Additional doses every 8-12hrs as needed. Consider multi-modal analgesia with NSAID and local analgesic.**
Non-steroidal anti-inflammatory analgesia (NSAID) -- Note that prolonged use my cause renal, gastrointestinal, or other problems			
Recommended: Carprofen	**4-5 SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hour**	**Depending on the procedure, may be used as sole analgesic, or as multi-modal analgesia with buprenorphine.**
Meloxicam	**0.1 – 0.3 PO, IM or SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 24 hour for up to 4 days.**	**Depending on the procedure, may be used as sole analgesic, or as multi-modal analgesia with buprenorphine.**
Ketorolac	**0.3 – 0.5 oral or SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hour**	**Depending on the procedure, may be used as sole analgesic, or as multi-modal analgesia with buprenorphine.**
Ketoprofen	**2 – 5 SC**	**Used pre-operatively for preemptive analgesia and post-operatively every 12-24 hour**	**Depending on the procedure, may be used as sole analgesic, or as multi-modal analgesia with buprenorphine.**
Local anesthetic/analgesics (lidocaine and bupivacaine may be combined in one syringe for rapid onset and long duration analgesia)			

Lidocaine hydrochloride	**Dilute to 0.5%, do not exceed 7 mg/kg total dose, SC or intra-incisional**	**Use locally before making surgical incision**	**Faster onset than bupivacaine but short (<1 hour) duration of action**
Bupivacaine	**Dilute to 0.25%, do not exceed 8 mg/kg total dose, SC or intra-incisional**	**Use locally before making surgical incision**	**Slower onset than lidocaine but longer (~ 4-8 hour) duration of action**

STANDARD OPERATING PROCEDURE #1

Sanitation - Animal Room Cleaning

Purpose: To set forth proper procedure to insure that animal rooms are maintained in a clean and sanitary manner.

Methods: All animal rooms are cleaned weekly. This cleaning includes the following:

1. All trash cans are emptied and a clean plastic liner inserted daily; containers are sanitized once per week.

2. Sinks and table tops are washed daily using a detergent disinfectant solution (Bio-Kleen) using one ounce in three gallons of water.

3. Rooms will be swept daily and mopped once or twice per week, or as needed.

4. All air vents will be cleaned weekly or as often as necessary.

5. Corridors are swept and mopped at least once per week, and walls and ceilings are cleaned once per month.

6. Room doors and windows are cleaned once per week.

7. Two rooms will be stripped of all equipment and will be washed down (including walls, ceiling, pipes, air ducts, and floors) each week, following removal of animals, and as needed. On this schedule, all rooms will be cleaned once per month. The room will be sanitized using a detergent-disinfectant solution.

NOTE: USE OF CLEANING AGENTS CONTAINING ODOR COUNTERAGENTS IS PROHIBITED.

STANDARD OPERATING PROCEDURE #2

Sanitation - Cage Cleaning Frequency

Purpose: To establish the frequency and the procedures for cage changing so that animals are housed in a clean environment.

Methods:

1. All small animals are transferred to clean cages at least once every two weeks, more often if needed.

2. Clean cages, outfitted in the same manner as the one with which they are exchanged, are brought into the animal room. In a methodical order, each animal and its cage are transferred.

3. Rabbit pans and cages are cleaned with Lime-Away de-scaler to remove urine deposits prior to cage washing.

4. Wire stainless steel tops are changed and sanitized at least once every two weeks.

5. Water bottles are changed at least once a week.

STANDARD OPERATING PROCEDURE #3

Sanitation - Rack Washing Frequency

Purpose: To establish procedures to assure racks are maintained in a clean condition.

Methods:

1. Racks in use are washed once each quarter; all racks are wiped down once a week; carts and racks are dusted daily or as often as necessary.

2. A detergent-disinfectant is used to sanitize racks and carts.

STANDARD OPERATING PROCEDURE #4

Operation of the Cage Washer

Purpose: To insure proper operation of the cage washer.

Methods:

1. Jets on impellers are checked daily and cleaned as necessary.

2. Cage washer will be cleaned out daily; both screens in the cage washer are cleaned daily.

3. After the cages have been dumped and rinsed, the cages are put into the cage washer.

4. Cycles for different types of cages are pre-programmed into the cage washer computer.

5. The desired cycle is selected and the machine is started.

6. After the cycle has been completed, the cages are placed on racks to dry. When dry, they are taken to the storage room, filled with bedding and stored for later use.

7. Quality Control reports are conducted monthly on a random sampling of water bottles and cages. The bottles/cages chosen are swabbed onto RODAC plates and then incubated to detect colony growth. The plates are incubated for 48 hours and the colonies are counted and recorded. Copies of the report are kept in the Animal Resource Facility Office.

8. All stainless steel in the cage wash room will be cleaned and polished once per week.

9. The cage washer is drained at the end of the day and the cage wash room is swept and mopped with a detergent-disinfectant at the end of every working day.

STANDARD OPERATING PROCEDURE #5

Bedding - Storage and Changing Procedures

Purpose: To insure proper storage and instructions for the proper use and disposal of bedding material.

Methods:

A. Receiving and Storage

1. Verify the amount received.

2. Refuse any bags that are torn and leaking. Patch immediately any bags torn during the unloading process or empty them into bedding container, provided that the bedding has not become wet or contaminated. Dispose of spilled or contaminated bedding. Do not accept wet bags.

3. Pallets for storage of bulk quantities are cleaned quarterly or more often as necessary with the high pressure sprayer. The bulk feed and bedding storage area is swept weekly.

B. Dispensing

Bedding is to be placed in clean animal cages only in the clean cage storage room. It is not to be done in the animal rooms. Filling of cages causes bedding particles to become dust particles in the rooms. The bedding can be placed in clean animal cages and placed in clean cage storage or bedding storage rooms.

C. Removal of Spent Bedding

After changing animals to clean cages containing clean bedding, the dirty cages containing dirty bedding are taken to the cage cleaning area. There the bedding is dumped into the Garbel disposal sink, the cage scraped, brushed, and sprayed out, and then placed on a rack for subsequent cleaning in the cage washer. If washing by hand instead of the cage washer, wash with water containing a detergent-disinfectant, rinse with hot clear water, allow to dry, and store in clean cage storage room.

STANDARD OPERATING PROCEDURE #6

Animal Feed - Monitoring and Storage

Purpose: To insure that animal feeds purchased by the ARF are received and stored until time of use in wholesome condition.

Methods: A. Receiving and Storage

1. Verify the amount and type of food. Check each lot for the date of manufacture. Inventory on a weekly basis, quantities on hand.

2. Refuse and return all bags that are:

   a. Torn and leaking food.

   b. Not stamped on the bag with a milling date (hand written milling date is unacceptable).

3. Patch immediately any bags torn during the unloading process or empty them into a feed container, provided that the feed has not been contaminated. Dispose of any spilled or contaminated feed, and bags which are wet or show evidence of having been wet.

4. Do not accept feed if more than two months have elapsed since manufacture or if older, mark each bag "Use by (date)" allowing six months from milling date, except guinea pigs food, which is three months.

5. Place accepted feed in the food storage room. Stack the feed on shelves in such a manner as to allow easy access to that food with the oldest milling date so it can be used first.

6. Rotate the older feed to the front and use prior to the new shipment.

7. Plastic container, lids, and roller base for storage of daily use food are sanitized prior to feed being placed into the container.

B. Feed Use

Technicians and animal care personnel using the feed are to check the general

appearance and odor of the feed and to watch for contaminants. Milling dates should be checked: guinea pig feed is not to be used if more than three months old; all other six months. Outdated or suspect feed is set aside and the Veterinarian notified.

C. Feeding

1. Animals on regular diets are fed daily, ad lib. Special diets provided by investigators can be given to the animals by the ARF personnel ad lib, in prepackaged units or in easily measured quantities. This applies to situations where very little, if any, records of consumption or spillage are required. Potentially hazardous agents will be fed by ARF personnel only after approval by the Animal Studies Subcommittee and Research Safety/Bio-Safety Subcommittee.

2. All feed bowls and hoppers are cleaned and sanitized in the cage washer once per week or more often if necessary.

STANDARD OPERATING PROCEDURE #7

Caging and Housing Dimensions for Laboratory Animals

Cage Dimensions	**Animals per Cage**
Rats in 19” X 10½” X 8” plastic cage	
Weight of Rat (grams)	Number in Cage
Up to 100	8
100 – 200	6
200 – 300	4
300 – 400	3
400 – 500	2
Over 500	1
Mice in 11½” X 7½” X 5” plastic cage	
Weight of Mice (grams)	Number in Cage
Up to 10	11
10 – 15	8
15 – 25	5
Over 25	4
Rabbits in 5 foot square stainless steel cage	
Weight of Rabbit (pounds)	Number in Cage
>11.88	1

STANDARD OPERATING PROCEDURE #8

Security Procedures

Purpose: To establish security procedures for the Animal Resource Facility.

Methods:

1. Unauthorized entry into the Animal Resource Facility is strictly prohibited. Special attention to physical security is warranted by the threat of property destruction and theft by groups opposed to the use of animals in research. The BBSC ARF is located on the ground floor of the Byrd Biotechnology Science Center on the campus of Marshall University. Security is maintained by "Authorized Personnel Only" signs on the entrance door. Doors are locked continuously and require a card swipe system to enter the outer doors and a PIN number to enter the inner doors and each animal room door. All exterior doors of the BBSC are automatically locked at 7:00 pm and unlocked at 7:00 am during work days. These doors are locked on weekends from 7:00 pm Friday until 7:00 am Monday, and all holidays. These doors require a card swipe to open during non-duty hours. Security personnel are in the building at all times and patrol the building during non-duty hours. Surveillance cameras utilizing computer hard drive storage are utilized in this facility.

2. Requests for tours of the ARF by the media and persons claiming to represent animal rights and animal welfare organizations must be approved by the Institutional Official. If approved, such tours will be conducted by the Director of the Animal Resource Facility.

3. Inquiries regarding lost pets should be handled with caution and sensitivity and will be referred to the Veterinarian.

STANDARD OPERATING PROCEDURE #9

Education and Training

Purpose: To outline the training program regarding the functions, operation, and use of the Animal Resource Facility and animal subjects; and to outline the Institutional Animal Care and Use Committee (IACUC) responsibilities regarding instruction available and assessment of the capabilities of investigators and staff to perform the investigation.

Methods:

1. Newly recruited staff are required to have training regarding the functions and operations of the Animal Resource Facility and use of animal subjects. Newly recruited animal care technicians undergo an intensive training program under the direction of the veterinarian. Work is closely, carefully, and frequently monitored. Each duty is explained and demonstrated. Reference material, Standard Operating Procedures, and the Policies and Procedures manual are current and are maintained in the ARF office for all employees who work with animals.

2. The training program includes, but is not limited to animal husbandry, sanitation procedures, AAALAC guidelines, Animal Welfare Act and other regulatory guidelines, guidelines for adherence to protocols, normal/abnormal behavior, signs/symptoms of illness, handling and manipulation of various species, records and reports, ethical considerations, including testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, occupational health and safety, and general operating guidelines for an Animal Resource Facility.

3. In addition to personal interaction with the veterinarian, audiovisuals, journals, bulletins, and magazines are used as training aids. The veterinarian also provides training and instruction to all research technical staff and investigators as appropriate.

4. The IACUC will assess the capabilities of each investigator and his/her staff as part of the review process before the study is permitted to begin. Specific areas of training include online training utilizing the CITI Program for Research Training. This training program is located at www.citiprogram.org. All new investigators are required to complete these courses prior to initiation of the use of animals in research. We require researchers to complete the course on Working with the IACUC. If there are any courses pertinent to the species the researcher is working with, they must complete those courses,

such as Working with Mice in Research Settings, Working with Rats in Research Settings, and Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress.

In order to complete the training component you should:

1) Go to the CITI site http://www.citiprogram.org/
2) log on if you have previously used this site or register as a new user.
3) The next point is to affiliate with an institution - (Marshall University) on a pull down menu
4) Next is to select the courses :
   a. Working with the IACUC;
   b. Working with Mice or Working with Rats, and
   c. Post-Procedure Care of Mice and Rats in Research
5) If you have previously completed training under reserachtraining.org then these certificates have migrated to CITI.org under previous coursework completed

STANDARD OPERATING PROCEDURE #10

Equipment Maintenance and Related Activities

Purpose: To establish an equipment and facility maintenance program for the Animal Resource Facility.

Methods: The following is the maintenance program governing the operation of the Animal Resource Facility.

Equipment used for animal experimentation is checked/inspected by Biomedical Engineering annually or more often if necessary.

The light cycle in each room is controlled by a centralized computer system and is set for 12 hours light and 12 hours darkness, unless the protocol requires a different cycle.

Macro environments are monitored by a centralized computer program which records humidity levels, intake air temperature, and exhaust air temperature.

The cage washer spray nozzles are checked daily and cleaned as needed.

The emergency power generator is inspected monthly by our Engineering staff and after each use.

Emergency Eyewash stations are checked on a weekly basis.

STANDARD OPERATING PROCEDURE #11

Veterinary Medical Care Program

Purpose: To outline the veterinary medical program of this facility.

Methods:

1. Veterinary care is provided by the attending veterinarian.

2. The veterinarian's major responsibilities are the appropriate and efficient functioning of the Animal Resource Facility (ARF), overseeing all professional aspects of the ARF program, including, but not limited to monitoring care and use of laboratory animals, establishment of policies and procedures for veterinary care, reviewing protocols, animal husbandry and animal welfare, monitoring occupational health and conducting educational programs for ARF staff and other research technicians.

3. His professional qualifications and interest lie in the specialty of Laboratory Animal Medicine and he serves the School of Medicine in all matters of Veterinary Science. The veterinarian is responsible for complying with and implementing all aspects of the "Laboratory Animal Welfare Act: (PL 89-544)" and its subsequent amendments. He stays informed in order to comply with future legislation that may affect the use of laboratory animals.

4. The veterinarian will serve on the Institutional Animal Care and Use Committee and inform the committee on matters dealing with laboratory animal care, use, etc. Veterinary care will be available for any investigator who desires this service for research or for which the IACUC mandates veterinary oversight. Consideration will be given to such things as:

    a. Suitable animals for the experimental design.

    b. Sources of animals and equipment.

    c. Necropsy results.

    d. Unusual environmental requirements.

    e. Special diets and care.

    f. Pre and post-operative care.

g. Special nursing care.

h. Analgesics and anesthesia.

I. Other professional, technical, and ancillary services.

5. Veterinarian Location

The Veterinarian is located in Room 112 of the Byrd Biotechnology Science Center and is immediately accessible by telephone. He provides training and guidance to the animal care technicians. Additionally, he meets with all investigators who submit research protocols utilizing animal subjects to discuss the study and animal species required. A back-up veterinarian is available to provide care in the absence of the veterinarian.

6. Primary Duties of the Veterinarian

The Primary Duties of the veterinarian include, but may not be limited to:

a. Directing the design and operation of the animal facility to ensure compliance with current animal welfare laws, regulations and policies, and to support R&D activities using animal subjects.

b. Providing professional guidance and technical support to the health care facility's investigators and technicians in planning, executing, and directing R&D activities using animal subjects.

c. Initiating or reviewing requests for equipment used in the animal facility and plans for animal facility construction and renovation.

d. Serving as a member of the Institutional Animal Care and Use Committee (IACUC). The IACUC oversees all animal research facility activities, including review of all protocols utilizing animal subjects, policies and procedures, physical facilities, and operations.

e. Reviewing all proposals that include the use of live vertebrate animals before consideration by the IACUC.

f. Participating in semi-annual inspections of animal facilities.

g. Contributing to the promotion of favorable community relations and

increased public appreciation of the importance of animal studies in improving patient care.

h. Consideration for humane aspects of animal experimentation such as the proper use of anesthetics, analgesics and tranquilizers.

i. Implementation of measures to alleviate unacceptable levels of pain or distress to animal subjects.

j. Provides training and guidance to animal care technicians and annual training of all staff. The veterinarian will conduct training sessions with the Animal Resource Facility Staff and with the research technicians and investigators for each new protocol or modification of an existing protocol, outlining the salient points of the protocol. This training will be conducted upon the receipt of the first shipment of animals and will be specific to each protocol or modification thereof.

STANDARD OPERATING PROCEDURE #12

Sanitation of Procedure Rooms

Purpose: To establish sanitation procedures for Procedure Rooms.

Methods:

1. Use of the Procedures Room must be scheduled in advance with Animal Care Staff.

2. All rooms will be swept and mopped with a cleaner/disinfectant and after every procedure and/or removal of animal(s).

3. All equipment will be cleaned once per week with a cleaner/disinfectant and after every procedure and/or removal of animal(s).

4. All tables (examining, etc.) and counter tops will be cleaned once per week and after every procedure and/or removal of animal(s).

5. All equipment will be removed once per month and walls, ceiling, pipes, and floors will be cleaned and disinfected with Bio-Kleen®, and after every procedure involving a large animal.

6. Trash will be emptied daily or as needed and new liners inserted. Trash containers will be sanitized once per week and after every procedure in the cage washer.

NOTE: ALL INVESTIGATORS WHO USE THE PROCEDURE ROOM ARE RESPONSIBLE FOR CLEANING UP AFTER THEMSELVES. ALL HEAVY CLEANING/DISINFECTING WILL BE PERFORMED BY ARF STAFF.

STANDARD OPERATING PROCEDURE #13

Operating Procedures for the High-Pressure Spray Cleaner

Purpose: To establish operating guidelines for the High-Pressure Spray Cleaner

Methods:

1. When animal rooms are stripped and cleaned, the walls and floor will be sprayed with the sprayer containing a cleaning/disinfectant solution

2. All stainless steel carts with hanging baskets will be sprayed with the sprayer using the same cleaning solution as is used in the animal rooms.

3. The sprayer is cleaned after each use.

STANDARD OPERATING PROCEDURE #14

Health Monitoring Program

Purpose: To establish a health monitoring program for animals housed in the Animal Resource Facility.

Methods:

1. The veterinarian is notified immediately when animals display signs of illness or injury.

2. When uncrating shipments of new animals, special attention is given to the level of activity of the animals and feces are observed for signs of illness. All animals are visually observed for gross lesions and/or symptoms of illness or disease each day.

    a. Every animal will be checked once a day for overt signs of illness and/or injury. This includes weekends and holidays.

    b. Any signs of unusual behavior, unhealthy appearance (not eating or drinking, diarrhea, lack of feces, bloody urine, etc.), or obvious signs of injury are immediately reported to the investigator or technician. The ARF personnel will then contact the veterinarian. The investigator and veterinarian will decide if treatment is warranted or if the animal should be euthanized. If disease is suspected in a dead or moribund animal, the veterinarian will conduct the examination or necropsy. At the discretion of the veterinarian, cultures or tissues will be taken for histopathology. If necessary, steps can also be taken to isolate the animal.

3. If an animal displays signs of illness or disease, it is immediately removed from the colony and quarantined. The veterinarian and Principal Investigator (PI) are contacted. The veterinarian examines the animal(s) and prescribes the necessary treatment or recommends euthanasia. Necropsy is performed and further diagnostic testing ordered if deemed necessary by the veterinarian or requested by the PI. If an entire colony displays symptoms of disease or illness, the entire colony is quarantined. If other animals are housed with this colony in the same room, the sick colony is removed and placed in quarantine.

4. During servicing of ill or suspected ill animals, ARF staff wears a gown, shoe covers, mask, and gloves. This procedure is followed when servicing animals

being held in quarantine as well. Upon removal of an ill colony (or suspected ill), the animal room is stripped and disinfected.

5. If an animal is unexpectedly found dead, a necropsy is performed and diagnostic testing is performed if deemed necessary by the veterinarian or requested by the PI.

6. All serology on animals will be done on recommendation of the veterinarian. Routine serology is done every six months on animals in long-term projects and breeding colonies. Serology for diagnostic purposes will be accomplished as deemed necessary in the professional opinion of the veterinarian. Serology is performed by an outside contract laboratory.

7. Diagnostic microbiology is performed by the VA Microbiology Laboratory. Pathology is performed by the Pathology Department at the Marshall University School of Medicine.

8. Health monitoring reports are obtained quarterly from all vendors from whom animals are purchased.

9. Microbiological cultures are performed monthly on random samples of cages and water bottles.

10. Virus Free and Non-Virus Free Animals: Specific guidelines for prevention of the spread of viruses to viral-free colonies are contained in paragraph #4 of this SOP. These include wearing a mask, gown, shoe covers, and gloves. These animals are serviced last each day. Protective clothing is discarded after leaving the room.

STANDARD OPERATING PROCEDURE #15

Guidelines for Aseptic Surgery on Rodents

Purpose: To establish guidelines for aseptic surgery on rodents.

The National Research Council's Guide for the Care and Use of Laboratory Animals states that "Survival Surgery on rodents does not require a special facility, but should be performed using sterile instruments, surgical gloves, and aseptic procedures to prevent clinical infections." As required by the U.S. Public Health Service and the Animal Studies Subcommittee all vertebrate animal-use protocols, for research or teaching, regardless of funding source, must comply with the guidelines stated in the Guide.

Listed below are guidelines, which are consistent with the Institutional Animal Care and Use Committee's interpretation of the N.R.C. guidelines and which provides satisfactory aseptic conditions. Investigators who feel that their vertebrate animal experiments require significant exceptions to these guidelines should contact the veterinarian for assistance. Otherwise, investigators will be expected to follow these guidelines.

Methods:

1. Surgery will be conducted on a clean, uncluttered lab bench or table surface. The surface will be wiped with a disinfectant before and after use and/or covered with a clean drape.

2. Hair should be removed from the surgical site with clippers or a depilatory. The surgical site should be treated with an antiseptic scrub and then with an antiseptic solution (chlorhexidine or povidone iodine solution).

3. All instruments will be sterilized. The method is determined by the surgical instruments or devices being used. Acceptable techniques for cold sterilization include soaking in 2% glutaraldehyde for ten (10) hours, in 8% formaldehyde, or in 6% stabilized hydrogen peroxide for six (6) hours.

4. The surgeon should wash his hands with an antiseptic surgical scrub preparation and then aseptically put on sterile gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have the first layer of the double-wrapped instrument pack opened before putting on sterile gloves.

5. The surgeon should wear a facemask. A cap and sterile gown are

recommended, but not required.

6. Multiple surgeries present special problems. After the first surgery, the sterilized instruments may be kept in a sterile tray containing or isopropyl alcohol. The alcohol should be replaced when contaminated with blood or other body fluids. Sterile gloves should be changed between surgeries if the surgeon touches the non-sterile surfaces, alternatively, surgeons may wipe their gloves for 30 seconds with sterile gauze pads soaked in isopropyl alcohol or non sterile surfaces may be handled aseptically with sterile gauze pads.

7. The abdominal or thoracic body wall should be closed with absorbable suture material. The skin should be closed with staples or with a non-absorbable suture material in a simple interrupted pattern. Skin sutures or staples should be removed in 7 to 10 days after surgery.

8. Rodents should be recovered from anesthesia in a warmed environment. Antibiotics may or may not be indicated after surgical procedures.

9. Access to the operating area should be restricted and the entire procedure should appear professional.

10. Analgesics should be administered prior to surgery and twice a day, as close to every 12 hours as possible, for 5-7 days after surgery.

Questions about aseptic surgical techniques may be addressed to the veterinarian.

STANDARD OPERATING PROCEDURE #16

Occupational Health

Purpose: To ensure that all employees who handle animals maintain in good health.

Methods:

A. Pre-employment physical and periodic screening examinations may be conducted. For animal caretakers and animal handlers, the physical may include a TB Skin Test, a Physical Examination by a physician, a Urinalysis, an EKG if indicated by age or health history, Blood tests: pre-employment screening blood tests may include a CBC and other tests as deemed necessary by the examining physician, a Tetanus Injection, a Rabies and other vaccinations as required by assigned duties. Personnel health records are maintained by the Occupational Health Office of Marshall University Medical Center. All Animal Resource Facility employees are vaccinated against tetanus and have annual physicals.

B. A risk assessment of employees will be performed utilizing the reference Occupational Health and Safety in the Care and Use of Research Animals. A questionnaire for risk assessment is attached as Table 1. "Marshall University Animal Handler's Health Questionnaire." These criteria will be used to determine the procedures to be performed on each employee to insure the Occupational Health Program is utilized appropriately. Accident reporting (including animal bites, etc.) is required and proper treatment and records are maintained by personnel.

C. Protective equipment such as caps, masks, gloves, gowns, scrub suits, restraining cages, mesh gloves, etc. are provided to all staff. Outer garments worn in animal rooms will not be worn outside the animal facility.

D. Injury prevention is an integral part of the orientation process for new employees and is routinely discussed at monthly staff meetings. Animal care technicians are instructed by the veterinarian in methods in handling animals, use of mesh gloves to prevent bites, and use of restraining cages for manipulations.

E. Experiments involving hazardous agents are monitored closely by the appropriate committees and the IACUC approval is not granted unless adequate facilities are available and appropriate safeguards are observed.

F. Other appropriate immunizations are available to Animal Resource Facility

personnel as determined by the personnel health physician, veterinarian, and nature of animal research projects.

G. All Animal Resource Facility employees receive initial training under the direction of the veterinarian regarding occupational health, including zoonoses. The education program regarding zoonoses, personal hygiene, and other considerations is under the direction of the veterinarian.

H. Transporting animals into or through areas used by visitors is to be avoided when feasible. When essential to do so, all reasonable means of minimizing health risks to visitors will be observed.

I. An eating and drinking area has been designated. Eating or drinking is not permitted in the Animal Resource Facility.

J. Smoking is not permitted within the MU BBSC.

K. Shower facilities are provided for animal care technicians.

L. Hand washing is mandatory upon entering and leaving any animal holding area or working with animal tissues.

Table 1

MARSHALL UNIVERSITY
ANIMAL HANDLER'S HEALTH QUESTIONNAIRE

Date: _____

Name: _____

Supervisor: _____

Department: _____

Age: _____ Sex: Male Female

OCCUPATIONAL HISTORY Answer these questions about your present job:

1. Job title: _____

2. Number of years employed at this facility: _____ years

3. How many months/years at your present position: _____

4. Description of duties (briefly): _____
_____

5. With which laboratory animals do you work?

Animal	Yes	No	Approximate contact hours/week
Rat			
Mice			
Gerbils			
Other			

6. Do you feel you are allergic to any animals? Yes No Please list those to which you feel you are allergic.
_____

7. Did you work with laboratory animals prior to employment at this facility? Yes No If yes, how long?_____ years What species of animals? _____

8. Do you use or wear any of the following items when working with animals?

	Yes	No
Protective eye glasses		
Mask/respirator		
Lab coat		
Gloves		

HOME ENVIRONMENT INFORMATION

9. Do you have any indoor pets? Yes No If yes, which animals and for how long?

Animal	1-2 years	3-4 years	over 4 years
Dogs			
Cats			
Other (List)			

10. Do you regularly have any of the following symptoms? Yes No
Please indicate if the symptom is present and the year of onset. Also check in what location or time "period" the symptom(s) is/are present.

Symptom	Present	Year of onset	Symptoms are present			
			At work	At home	On vacation	No difference
Cough						
Sputum production						
Shortness of breath						
Wheezing						
Chest tightness						
Asthma						
Nose congestion						
Runny nose						
Sneezing						
Itchy eyes						
Sinus problems						
Hay fever						
Frequent colds						
Hives						
Skin rash						
Swelling -eyes/lips						
Eczema						
Difficult Swallowing						

Immunization History:

Vaccine	Yes/No	Date	Vaccine	Yes/No	Date
Hepatitis B			Tetanus-Diphtheria		
Hepatitis A			Rabies		
Comments:					

11. Were you ever told by a doctor that you had allergies? Yes No

12. Have you ever been skin tested for allergies? Yes No If so, to what substances were you found to be allergic or sensitized? _____

13. Have you ever received allergy (desensitization/immunotherapy) shots? Yes No

14. Has a doctor ever said you have asthma? Yes No

If yes, when did your asthma start? _____(year)

Are you currently taking medication (either over the counter or by prescription) to control your asthma? Yes No

15. Has a doctor ever told you that you have a medical condition caused by your working conditions? Yes No

16. Do any of your blood relatives (grandparents, parents, brothers/sisters) have allergies or asthma? Yes No

17. Are you under a doctor's care for any other illnesses? Yes No If yes, please list illnesses (optional - however, if you are immunosuppressed due to treatment or illness, please let Occupational Health and your supervisor know so that proper protective equipment can be provided in accordance with your physician's recommendations).

18. Do you regularly use "over the counter" (non-prescription) nose drops or nose sprays, e.g. Afrin, Neosynephrine? Yes No

Comments: _____

_____

Further evaluation needed by physician: NO - YES -

Additional Personal Protective Equipment (PPE's) needed (beyond lab coats and gloves):
NO - YES -
Recommended additional PPE's: _____

Reviewed by: _____ Date: _____

OCCUPATIONAL HEALTH QUESTIONNAIRE WAIVER

At this time, I decline to participate in the Occupational Health Questionnaire for persons having contact with animals.

_____

Signature Date

I wish to have my personal physician administer my Occupational Health Program

_____

Signature Date

Laboratory Animal Allergies

Why it's important:

When working with animals it is important to be aware of the risk of developing allergies to the animals you work with or even to your own pets. If you become allergic to the animals you work with, your job can become quite uncomfortable and even unhealthy. If you have asthma, working with animals to which you are allergic can be a significant health risk.

Symptoms:

Allergy symptoms can range from minor to severe. People who are having an allergic reaction can get a runny nose, runny or itchy eyes, asthma (characterized by wheezing and shortness of breath), a skin rash or bumps, or even gastrointestinal (GI) disorders. It is important that you notify Employee Health if you have any of these symptoms that last more than a few days or if your symptoms are severe.

How it happens:

People who work with or even near animals can be allergic to any animal species. The allergens are proteins that are found in animal body fluids and skin. These substances can stick to animal hair and dust particles and can float around in the air. Allergens are unique in each animal species. That's why a person can be allergic to mice but not rats or cats but not dogs. It's also possible to be allergic to more than one species. In fact, if you are allergic to something (an animal species or anything else) you're more likely to become allergic to something new than a person who isn't allergic to anything.

Because people can become allergic to any animal species, you may become allergic to the species you work with or any other species that is housed in the ARF or is taken to a lab nearby yours.

Relative Risk:

The incidence of animal allergies among people who work with animals may be as low as 10% or as high as 30%. This means that most people who work with animals are not allergic to them. But, this also means that if you work with animals your risk of allergy to them is as much as three times higher than people who don't work with animals.

Prevention:

The most important part of preventing animal related allergies is to minimize exposure to animals as much as possible. The following practices may help you to reduce your exposure to animals:

- Work with your animals in a ventilated hood or Biosafety cabinet when possible.
- When not working in a hood, make sure that the room where you're working with animals is well ventilated. Contact the Engineering Department if you have any questions about the air handling in your lab.
- Don't wear your street clothes when working with animals. Always wear a lab coat or scrubs, or even a disposable gown or jumpsuit.
- Don't take your lab coat or scrubs home for washing. See your supervisor about where to launder lab coats and scrubs.
- Always wear gloves when handling small animals.
- Wash your hands frequently and always after handling animals.
- Avoid touching your face when working with animals.
- Keep your work area clean.
- Be considerate of others - keep animal cages properly covered when moving them through common hallways. Do not touch common items (such as door handles) with animal-use gloves or unwashed hands.

Treatment:

If you think you're allergic to animals you work with or around, contact Occupational Health at 691-1178. Your symptoms may be controlled by medication or increased measures to reduce exposure. Also speak with your own physician about your allergy symptoms. Some people have such severe symptoms they cannot continue to work with or near animals.

If you require personal protective equipment to protect yourself from animal allergens, notify your supervisor and have him/her contact the safety office.

STANDING OPERATING PROCEDURE #17

MARSHALL UNIVERSITY SCHOOL OF MEDICINE
ANIMAL RESOURCE FACILITIES
DISASTER PLAN

The following has been prepared as a guide for handling an emergency in the Animal Resource Facility that places the health and well-being of research animals at risk.

Emergency/Disaster During Regular Duty Hours

Employees will follow existing procedures for emergency/disaster during regular duty hours. Any and/or all animals will be rescued if possible, without endangering human life or health. For animals housed in the BBSC, safe areas will be utilized for evacuation sites. If there are no safe areas in the BBSC, attempts shall be made to evacuate animals to the Huntington Veteran Affairs Medical Center Animal Resource Facility if the VAMC is capable of accepting these animals. If no suitable areas are available, the animals will be monitored for injury or distress and, if needed, be humanely euthanized. Animals that are injured or in distress will be humanely euthanized by the means appropriate for that species, in accordance with the AVMA Guidelines on Euthanasia. Care will be supervised by the ARF Director.

Emergency/Disaster After Regular Duty Hours

Responders will follow existing procedures for emergency/disaster after regular duty hours. Any and/or all animals will be rescued if possible, without endangering human life or health. For animals housed in the BBSC, safe areas will be utilized for evacuation sites. If there are no safe areas in the BBSC, attempts shall be made to evacuate animals to the Huntington Veteran Affairs Medical Center Animal Resource Facility if the VAMC is capable of accepting these animals. If no suitable areas are available, the animals will be monitored for injury or distress and, if needed, be humanely euthanized. Animals that are injured or in distress will be humanely euthanized by the means appropriate for that species, in accordance with the AVMA Guidelines on Euthanasia. Care will be supervised by the ARF Director.

Emergency Telephone Numbers

	Office	Home
Billy W. Howard, DVM ARF Director	696-7374	654-9817
Monica Valentovic, Ph.D Chairman, IACUC	696-7332	697-0321
Jan M. Ball, DVM Backup Veterinarian	525-5121	429-5148
Tara Runyon Animal Technician	696-7373	525-6867
Lonny Muncy Animal Technician	696-7373	525-5220

STANDARD OPERATING PROCEDURE #18

Prolonged Restraint Policy

PURPOSE: This policy establishes procedures for prolonged restraint of any animal.

METHODS: Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished naturally or with devices such as restraint stocks or squeeze cages. It is important that such devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Prolonged restraint of any animal should be avoided unless essential to research objectives and requires approval of the IACUC. The following are important guidelines for the use of restraint equipment:

- Animals to be placed in restraint equipment should be conditioned to such equipment prior to initiation of the research.

- The period of restraint should be the minimum required to accomplish the research objectives. Prolonged restraint for any reason must be approved by the IACUC.

- Restraint devices are not to be considered "normal" methods of housing, although they may be required for specific research objectives.

- Restraint devices must not be used simply as a convenience to investigators in handling or managing animals. When such devices are used, their use must be specifically approved by the committee.

- Attention must be paid to the possible development of lesions or illnesses associated with restraint, including contusions, decubital ulcers, dependent edema, and weight loss. If these or other problems occur, veterinary care must be provided to treat the animal, which if necessary must be temporarily or permanently removed from the restraint device.

STANDARD OPERATING PROCEDURE #19

Multiple Major Surgical Procedures

PURPOSE: This policy establishes procedures for multiple major survival surgical procedures on a single animal.

METHODS: Multiple major survival surgical procedures on a single animal are discouraged. However, such procedures may be performed with the approval of the IACUC after careful deliberation of potential benefits and assurance of minimization of stress to animals.

The procedure for securing approval of multiple survival surgeries is the same as for any other protocol, with the following additional requirements: the investigator must attend the IACUC meeting and thoroughly explain his/her protocol to the full Committee. He/She must present and defend the need for multiple survival surgeries. Cost savings alone is not an adequate reason for performing multiple survival surgical procedures.

STANDARD OPERATING PROCEDURE #20

Procedures for Handling Allegations of Mistreatment or Noncompliance Involving Care and Use of Animals

PURPOSE: One of the basic functions of the IACUC is to review and, if warranted, investigate concerns involving the care and use of animals at the facility resulting from public complaints received and from reports of non-compliance received from laboratory or research facility personnel or employees. This policy establishes procedures for reporting, receiving and handling allegations of animal mistreatment or other noncompliance.

METHODS: a. Definitions

Mistreatment: Any action, physical or psychological, which results in wrongful or abusive treatment of an animal (e.g., inadequate or improper care or housing of animals).

Noncompliance: Violation of University procedures or policies, which encompass those of the Public Health Service and the Animal Welfare Act. Examples include unauthorized use of animals for an activity or procedure; failure to have an active IACUC approval for an activity involving animals; continuing an activity past its authorized expiration date.

Reporting allegations: It is not always obvious at what level of alleged mistreatment or noncompliance the IACUC should become involved. Frequently the attending veterinarian, animal care personnel, and investigators can work together to prevent or resolve a problem. However, serious or repeated problems always require the involvement of the IACUC. If in doubt it is better to submit a report, as this may well protect the institution, the complainant, the alleged violator(s), and, of course, the animals.

b. Reporting Procedures

Alleged allegations may be reported in conversation with, or correspondence (letter, FAX, email) with members or staff of the IACUC, the veterinary staff, or the Institutional Official (President of the University). There shall be no restrictions on who can report an alleged incident and there can be no threat of reprisals against anyone reporting the perceived mistreatment or

noncompliance. Any information should be quickly relayed to the Chairperson of the IACUC for action. All complaints brought to the attention of the IACUC will be fully documented under signature. There must be sufficient substance to the complaint for the Chair to proceed further. An allegation has no substance until proven, and should remain confidential to the extent possible to protect all concerned. If the complainant has freely identified him/herself, it is appropriate that receipt of the allegation be acknowledged.

c. IACUC Procedures for the Investigation of a Complaint

The Chairperson may elect to bring the matter as a whole before the committee or may appoint a subcommittee to investigate the complaint. In either case the results of the investigation must be considered IACUC actions and all members must have the opportunity to present their views. The person(s) against whom the complaint has been raised should have an opportunity to explain their position. As much documentation as is reasonably needed will be collected. This may include animal receiving records, housing and health records, billings, memos, and other written materials. It may also be necessary to interview persons or to carry out an inspection of the facilities. The results should be made available to all parties involved, including the Institutional Official who is ultimately responsible for instituting corrective action.

d. Institutional Responses

This is influenced by legal requirements, institutional policy and the nature of the investigative findings. If the violation is verified by the IACUC, the IACUC is empowered under USDA Regulations and PHS Policy to suspend a previously approved project. If the activity is supported by PHS funds, the IACUC, through the Institutional Official, must file a full report to the National Institutes of Health, Office for Laboratory Animal Welfare (OLAW). In cases where there is sufficient evidence of serious noncompliance, it may be prudent for the IACUC to suspend an activity pending the outcome of a full investigation. In these cases, a preliminary report will be sent to OLAW and the USDA, through the Institutional Official, on the understanding that a full report will be submitted upon completion.

The Institutional Official, in consultation with the IACUC, has the power to impose further sanctions on an investigator found to be responsible for

mistreatment or noncompliance. Each case must be considered individually and may result in precedents being set, therefore, the implications of these should be considered. The institution must also consider whether to announce its findings publicly.

Standard Operating Procedure #21

RODENT BLEEDING POLICIES

PURPOSE: In many experimental protocols, it is necessary to obtain blood from rats, mice, gerbils, hamsters, and guinea pigs. The Marshall University School of Medicine Institutional Animal Care and Use Committee endorses the following techniques as acceptable means for routine blood collections (species variations are noted).

POLICY:

A. Cardiac Puncture

This technique is generally employed when a larger quantity of blood is necessary in a short amount of time. However, it is a relatively difficult technique that is best performed by experienced, trained personnel. Also, it is a technique to be performed on anesthetized rodents only. Due to the possibility of causing cardiac tamponade and subsequent death, it is generally best employed as a terminal procedure.

B. Retro-orbital (Eye) Bleeding

In this procedure, the orbital sinus of rats, mice, hamsters, or gerbils is punctured in order to obtain a small blood sample. This procedure requires the animal to be anesthetized. When properly performed on the anesthetized animal, the eye is not damaged.

However, the eye will be damaged when repeated bleeds are done using the same eye frequently. Therefore, at least two weeks should lapse between bleedings from the same eye. A bland ophthalmic ointment should be applied at the end of the procedure to prevent corneal drying. Pressure over the orbital sinus after the bleeding tube is withdrawn will prevent further bleeding complications. This technique is not recommended in guinea pigs.

C. Venipuncture

Direct access into a vein is a common method for both blood collection and drug injection. The site of venipuncture will vary according to the species.

In mice, tail vein and jugular vein bleeding are the best choices. Proper restraint is essential for collection from both sites. Tail vein bleeding is facilitated by transillumination of the tail, warming the tail and/or the whole animal, occlusion of the veins at the base of the tail, and/or applying oil of winter green or alcohol. Xylene/Xylol is not recommended because of the skin irritation and slough that they can cause. For jugular vein bleeding, proper animal positioning is crucial in a conscious mouse, some hair clipping is necessary, and occlusion of the opposite jugular vein facilitates blood collection. A 27 gauge or smaller needle is recommended for mouse venipuncture.

Rats, in addition to the mouse sites and techniques noted above, can also be bled from the dorsal metatarsal vein or the saphenous vein (requiring a hair clip, aseptic preparation and a 27 gauge or smaller needle), the sublingual vein which requires anesthesia and a 26 gauge needle. For tail bleeding, a 19 gauge or smaller needle is recommended.

At any venipuncture site, direct pressure applied to the site after the needle is withdrawn, is required to control hematoma formation. If venipuncture needs to be repeated on a frequent basis (whether for blood collection or injection), alternative routes of bleeding/injection should be considered, including implantation of a chronic indwelling intravenous catheter.

D. Decapitation

This technique can be used to collect blood from smaller rodents. A guillotine or autopsy shears are used to perform the decapitation.

This technique should be used in research settings only when scientifically justified by the user and approved by the IACUC. Personnel employing this technique should receive appropriate preparation and training from a person experienced in this technique.

Blood collections in this manner will be contaminated with tracheal and salivary secretions.

This method is commonly used to collect blood from neonatal rodents.

E. Exsanguination

This is a terminal procedure that must be carried out under surgical anesthesia.

Exsanguination can be carried out in a variety of ways, i.e., cardiac puncture, severing of major blood vessels, aspiration of the major blood vessels with a syringe and needle, etc. Verification of the rodent's death is necessary before disposal of the carcass. This may be done by: physical means, i.e., decapitation, thoracotomy, cervical dislocation; chemical means, i.e.,CO_2, barbiturate overdose; and/or by observation of cessation of heart and lung movements.

F. Other Methods

Other methods of rodent bleeding may be acceptable, i.e., tail snips, which will be considered on an individual basis by the veterinarian and approval by the IACUC. The ARF Director should be consulted during the formulation of the research plan if there are any questions regarding proper blood collection techniques.

Additionally, consideration should be given to total blood volume withdrawn within a given time frame. Over-bleeding can leave animal hypovolemic, anemic, and weak. As a rule, up to 25% of total blood volume can be collected in a two-week period with replacement fluid (sterile 0.9% saline) recommended for larger collections.

Standard Operating Procedure #22

IMMUNIZATION OF RABBITS WITH COMPLETE FREUND'S ADJUVANT

General Background

Immunization with Freund's complete adjuvant (FCA) is a widely used procedure to stimulate antibody production. It is often associated with undesirable side effects such as granuloma formation, tissue necrosis and sloughing, abscessation and fever.

These lesions are generally caused by:

1. Instillation of too much inoculum at the injection site.
2. Injection sites which are too close together.
3. Use of improper injection sites.

Undesirable local lesions can be prevented by carefully limiting the quantity injected at each site and widely scattering the injection sites to ensure adequate barriers of normal skin. Trials have shown that using 1:1 suspensions of FCA and aqueous antigens, intradermal (I.D.) injections of 0.05 ml per site and subcutaneous (S.Q.) injections of 0.1 ml per site in rabbits produce palpable lumps that can be monitored for complications.

The limited quantity and scattering sites requires more time because more of the back and flanks must be clipped and prepared for injection, and more training and skill to instill small quantities of innoculum. However, an advantage of higher antibody production is gained by broader area of stimulation reacting with more regional lymph nodes and by the creation of many small foci of local cell interactions. The favorable results more than compensate for the greater time and skill required.

The peritoneal cavity and the footpad are considered improper sites for the use of FCA. These sites are determined as not acceptable.

FCA is considered a human biohazard, since accidental self-inoculation or splashing in the eye has been shown to cause painful sequelae not readily amenable to treatment, as well as sensitization to tuberculin.

Immunization Procedures

The majority of rabbits can be immunized using only manual restraints since the inoculation causes only minor or transient pain. The back, forequarters and flanks of the rabbit should be clipped using

a #40 surgical clipper head. This allows clear visualization of the injection sites and a clean work field. Loose hair should be brushed away and the skin prepared for injection using 70% alcohol. A rabbit restrainer is not generally used since the injection field covers more surface area than is generally accessible through the restraint devise.

Freund's Complete Adjuvant is mixed 1:1 with a suspension of the antigen and is used for the initial immunization only. Each subsequent booster injection will be done with Incomplete Freund's Adjuvant (IFA). The initial immunization may be a total of 2.0 ml (1.0 ml of FCA plus 1.0 ml of antigen). The adjuvant and antigen are mixed into a cloudy suspension using two syringes and a double-headed single-barreled micro-emulsifying needle.

A mixture of intradermal and subcutaneous injection sites may be used with no more than 0.05 ml of suspension inoculated I.D. at each site and no more than 0.1 ml of suspension inoculated S.Q. at each site. Be sure to allow at least 2 centimeters between I.D. sites. Best results are obtained using a 23 gauge 1 inch needle with the bevel up. This is generally tolerated very well by the rabbits with minimal signs of discomfort. It is recognized that the large number of sites involved and the small amount of inoculum delivered requires more time, a larger field and better technique control than most standard inoculation protocols. The favorable results, reflected in the high titers of antibody produced and the clinical well-being and appearance of the rabbits, fully justifies the additional time and care required.

The following schedule is typical of those used in the Neurology Institute (NINCDS) at NIH for producing antibody to synthetic peptides; schedules may vary depending upon the antigen.

Week #1 -- Initial immunization with Freund's Complete Adjuvant (FCA).

Week #3 -- Boost with Incomplete Freund's (IFA).

Week #5 -- Boost with Incomplete Freund's (IFA).

Week #6 or #7 -- Bleed to check serum titer (usually 5 ml), if titer is favorable, follow with a larger bleed.

Follow this with monthly boosts with Incomplete Freund's Adjuvant, bleeding three or four weeks after the boost for 25 to 30 ml.

Current practices in humane care recognize that these animals are monitored closely for reactions by the ARF staff and the Principal Investigator. Reactions will be treated by current accepted veterinary medical procedures. Rabbits that develop lesions that do not respond to treatment may have to be euthanized.

Bleeding Rabbits

A maximum of 1% of body weight may be collected every three weeks. (For example, 25 cc of blood could be collected from a 2.5 kg rabbit.)

Blood may be collected by cannulation of the marginal ear veins or central ear artery. Cardiac puncture should be reserved for terminal procedures, and requires a surgical plane of anesthesia.

Using tranquilizing agents to relax the rabbit and promote vasodilation is advised. Acepromazine tranquilizer at 1.0 mg/kg S.Q. prior to blood collection is one method.

Application of Xylene to dilate ear vessels is not recommended. This substance may cause scarring of the ears.

Standard Operating Procedure #23

Immunocompromised Animals

A. General

1. Entry into the animal rooms containing immunocompromised animals is restricted to ARF personnel and the Principal Investigator and staff.

2. Entry should be as infrequent as possible and the immunocompromised animal room should be entered first, before any other procedures are performed in the ARF.

3. An isolation gown must be worn in the immunocompromised animal room and removed as you leave. This gown will be worn in this room only.

4. Autoclaved cages and bedding, water bottles and water, and any special husbandry materials will be provided by the Principal Investigator and staff.

B. Cleaning

1. The immunocompromised animal room and everything in it will be thoroughly cleaned after each group of animals is removed.

2. The species of animals housed in the immunocompromised animal holding rooms determines the cleaning schedule. These schedules will be the same as immunocompetent animals of the same species, unless a different schedule is agreed upon by the Veterinarian and Principal Investigator.

Standing Operating Procedure #24

MANAGING IMMUNOCOMPROMISED ANIMALS

A. General

This SOP describes methods for maintaining immunocompromised rodents to prevent infections from adventitious pathogens.

B. Methods

1. Preparing the microisolation housing unit.

    a. Assemble the microisolation housing unit by placing bedding into the cage, placing the wire bar lid, water bottle, and the filter top on the cage.

    b. Autoclave the assembled microisolation housing unit in a vacuum autoclave set to the appropriate gravity setting.

    c. Autoclave a flask filled with water on the liquid cycle to fill the bottles at the time they are put in use.

2. Preparing the operator and workbench

    a. Put on a disposable lab coat and gloves. Pull the gloves over the cuff of the lab coat and tape to form a tight seal.

    b. Place the autoclaved microisolation housing unit, water flask, and animals near the laminar-flow workbench.

    c. Spray arms and gloves with disinfectant. (Gloves should be damp with disinfectant at all times while working in the workbench.)

    d. Wipe down the internal surfaces of the workbench with disinfectant and paper towels. Allow disinfectant to sit for three minutes.

3. Load the microisolation housing unit.

    a. Place the autoclaved microisolation housing unit in the workbench and remove the filter top. Place the filter top upside-down on the surface of

the workbench. (This procedure prevents dust particles from blowing from the top of the filter top into the cage bottom.)

b. Spray the water flask with disinfectant and place in the workbench. Fill the water bottle, add the stopper with sipper tube, and place in the cage.

c. For filter-crated animals (obtained from a specific pathogen-free colony), inspect the crate carefully for any holes or breaks in its integrity. If completely intact, thoroughly spray the crate (including the filter) with disinfectant and place in the workbench. Open the crate. If a tool is used, it must be kept in a tray containing disinfectant in the workbench.

d. If animals are currently in a microisolation unit, place the unit in the workbench and open as in 3.a. above. Do not spray the cage or cage filter.

e. Take a pair of totally submerged rubber-tipped forceps from the forceps tray in the workbench and shake off excess disinfectant in the hood. Lift the wire bar lid, grab animals by the tail, carefully transfer to the cage, and replace the filter top on the cage bottom. The unit is now ready and can be placed on the animal rack

f. Spray and wipe workbench with disinfectant after all animals have been transferred to clean microisolation units and placed on the animal rack.

g. Remove soiled microisolation units to the cage wash room and used shipping crates to the dumpster.

STANDING OPERATING PROCEDURE #25

PROCEDURES FOR THREATENING SITUATIONS FOR ANIMALS

A. General:

This SOP describes procedures to follow during situations where animal health and safety is threatened.

B. Methods:

1. Whenever a threatening situation, other than fire, is discovered (temperatures 80ºF or above or 64ºF or below, lack of air flow, etc.) the Veterinarian and the Maintenance Section Chief will be contacted. If there is no response from the Veterinarian and/or the Maintenance Section Chief, the Animal Care Technicians and the alternate Maintenance Section designee will be contacted. These individuals will assess the situation (i.e., check animal room temperatures and air flow) and take corrective measures to insure the animal health and safety is not compromised.

2. The Veterinarian will compile a list of emergency telephone numbers from each investigator. This list will be maintained in the Veterinarian's office and the Animal Care Technicians' office.

3. Emergency telephone numbers for the Veterinarian, Animal Care Technicians, and Maintenance Section will be posted on the bulletin board outside the Animal Care Technicians' office, room 110, and in the Maintenance Section office.

4. The Principal Investigators will be notified by the Veterinarian of the situation and what immediate corrective measures are being taken. The Veterinarian will answer any questions the Investigator may have concerning the safety of their animals and the research implications involved. The Principal Investigator will then apprise the Veterinarian of any alternative measures their animals may require.

5. The Veterinary and Maintenance staffs will remain on location until the situation is resolved.

6. This SOP will be posted on the bulletin board outside room 110 in the ARF and should be used as part of any orientation for all new animal users.

Emergency Telephone Numbers

	Office	Home
Billy W. Howard, DVM ARF Director	696-7374	654-9817
Monica Valentovic, Ph.D Chairman, IACUC	696-7332	697-0321
Jan M. Ball, DVM Backup Veterinarian	525-5121	429-5148
Tara Runyon Animal Technician	696-7373	525-6867
Lonny Muncy Animal Technician	696-7373	525-5220

STANDING OPERATING PROCEDURE #26

RODENT BREEDING COLONY MANAGEMENT

A. General

This SOP describes procedures to follow for the management of rodent breeding colonies.

B. Methods

1. The cleaning and sanitation frequency for rodent breeding colonies will be the same as for rodent holding rooms.

2. The Principal Investigator will be responsible for setting-up the mating scheme and breeding scheme to insure the resulting offspring are as expected, i.e., maintaining either an outbred or inbred colony or if a mutant or transgenic, that the parents carry the desired mutation or transgene.

3. Sick or poor-doing neonates will be euthanized utilizing halothane as the euthanatizing agent, or by physical means complying with the current recommendations of the “AVMA Guidelines on Euthanasia.”

4. After the age of 4 weeks, the neonates will be counted as adults for census records.

5. The majority of “normal” offspring will be euthanized. Several of these offspring will be utilized as sentinel animals. They will be kept until they are four months of age, bled for serology testing, euthanized, and gross necropsy performed. Tissues will be collected for histological examination if any appear abnormal.

STANDING OPERATING PROCEDURE #27

SENTINEL ANIMAL MANAGEMENT

A. General

This SOP describes procedures to follow for the management of sentinel animal cages.

B. Methods

1. The cleaning and sanitation frequency for sentinel animals will be the same as for rodent holding rooms.

2. The sentinel animal cage will be the last cage changed on the rack.

3. When changing other animals on the rack, a small amount of dirty bedding from these cages will be placed into the cage to be used for the sentinel animals.

4. After the sentinel animals have been placed into the new cage, this cage will be placed closest to the exhaust plenum (upper right slot).

STANDING OPERATING PROCEDURE #28

Cage Card Information

A. General

This SOP describes procedures to follow for the completion of the animal cage cards. This information is used to provide accurate identification for all animals.

B. Methods

1. Upon entry into the vivarium a cage card will be completed for all animals.

2. The information completed on the card is as follows:

a. Species of animal(s)
b. Sex of animal(s)
c. Weight of animal(s)
d. ID number (if has collar, ear tag, etc.)
(1) Rodents and lower species may or may not be assigned an individual identification number.
(2) Species above rodents will be assigned an individual identification number.
(3) If an identification number was assigned by the vendor, this number will continue to be used by SOM vivarium.
e. Account number the animals are charged to (i.e. grant number or department account).
f. Date animal(s) was (were) received
g. Source of the animal(s)
h. The number of animals in the cage.
i. Investigator's name
j. Investigator's department
k. The IACUC assigned protocol number

3. The cage card is placed on the animal's cage.

4. As animals are removed from or added to the cage, the number of animals marked on the cage card will be changed by the person who adds or removes the animal(s) from the cage.

STANDING OPERATING PROCEDURE #29

Euthanasia Chamber

A. General

This SOP describes procedures to follow for euthanizing small rodents using carbon dioxide.

B. Methods

1. Only medical grade carbon dioxide from a gas cylinder will be used.
2. Insure the chamber is empty and clean.
3. Place the animals into the chamber, insuring the animals are not overcrowded.
4. Open the valve on the cylinder.
5. If using a **MOUSE** cage adjust the flow rate to **2 lpm** on the regulator.
6. If using a **RAT** cage adjust the flow rate to **5 lpm** on the regulator
7. After observing no signs of breathing or movement from the animals, remove the animals. Insure the animals have been euthanized by applying cervical dislocation or bilateral thoracotomy.
8. Close the valve on the cylinder.
9. Place the animals into a bag and remove them to the cold room for disposal.
10. Clean and disinfect the chamber.

STANDING OPERATING PROCEDURE #30

TAIL SNIPPING IN MICE

PURPOSE:
To describe procedures used to obtain tissue needed for transgene identification in mice.

APPLICATION:
This procedure applies to colony breeders, researchers and technicians who collect tissue samples from mice for transgene identification.

NOTES:
PCR techniques may require less tissue and allow use of auricular flap tissue obtained during the ear punch identification procedure. Southern Blot testing may require more material and need tissue from the tail.

Standard Techniques:

Ear Punching: Ear punching does not require anesthesia in mice thru 21 days of age. Several tissue samples approximately 0.5 mm in diameter can be obtained.

Tail Snipping: Anesthesia is not required in mice through 21 days of age if less than 1 cm of skin is taken (skin can be pushed down toward the tip of the tail so that the vertebrae are avoided). Innervation of the tip of the tail is minimal at this age (Pain Category B).

Tail tip samples greater than 1 cm in length will probably damage the coccygeal vertebrae and will require anesthesia in mice of any age. Anesthesia is required for any tail snipping if animals are greater than 21 days old (Pain Category C).

Anesthesia:

Local:	FluorEthyl™, cetyl chloride, or similar hypothermic methods.
Injectable:	Ketamine Hydrochloride/Xylazine mixture
Inhalant:	Inhalant anesthetics (halothane or isoflurane, with appropriate safety precautions for personnel) or CO_2

PROCEDURES FOR TAIL SNIPPING

Gloves should be worn when handling laboratory animals

Anesthetize mouse (if required).
Gently, but securely, restrain mouse.
Swab tail with alcohol (povidone iodine or chlorhexidine solutions may interfere with the DNA identification tests).
Push skin toward tip of tail.
Snip skin sample (with sterile instrument[s]).
Cauterize and/or apply gentle compression until hemostasis occurs.
Release mouse.
Observe mouse for bleeding or abnormal behavior.
Check tail daily to ensure tip is healing.

STANDING OPERATING PROCEDURE #31

MOVEMENT OF ANIMALS

PURPOSE:
To describe procedures used when animals are removed and returned to the ARF.

APPLICATION:
This procedure applies to all animal users in the BBSC.

METHODS:

1. Whenever animals are removed from the ARF they will be transported in cages with lids securely in place.

2. If transporting animals outside the building, cages should be covered to avoid the general population from viewing the animals.

3. If transporting animals outside the building, they should be transported in a climate-controlled vehicle.

4. When the animals are returned to the ARF, they should be brought to the rear door of the animal room via the dirty corridor.

5. When the animals are moved into the animal room, the cages will be sprayed with disinfectant prior to being replaced into the rack.

STANDING OPERATING PROCEDURE #32

Receipt of Animals

A. General

This SOP describes procedures to follow for receipt of rodents into the animal resource facility.

B. Methods

1. Animals will enter the facility through the receiving area.

2. From the receiving area the animals will be delivered through the dirty corridor to the rear door of the assigned animal room.

3. The technician responsible for checking the animals and putting them into cages will open the rear door of the animal room and spray the shipping crate with disinfectant prior to bringing the crate into the room.

4. The crate will be placed in the laminar flow workbench, opened, and the animals checked for any abnormalities.

5. The animals will then be transferred into cages to be placed onto the rack.

6. Receipt of animals will be noted on the census sheet and on the health record.

STANDING OPERATING PROCEDURE #33

Minimum Protective Clothing for Animal Care/Use Areas

A. General

Protection of the health of all humans who work in animal care or use areas is critically important. A minimum standard for clothing has been established which is intended to discourage contamination of “non-work” clothing. This is not a new requirement; it is an enhancement of the existing policy consistent with occupational health and safety standards for work with laboratory animals.

B. Methods

1. All research staff must assure that the minimum clothing requirements are maintained for all members of the research group entering into animal care or use areas.

2. All support staff will also adhere to the minimum clothing requirements for entering into or working within the animal care or use area.

3. The ARF will develop additional policies for appropriate personal protective equipment (PPE) upon the advice of the Special Programs Coordinator for occupational health and safety.

4. A lab coat, gown, scrubs, or specified work uniform must be worn when entering animal occupied areas.

5. Gloves are recommended at all times when working with animals.

STANDING OPERATING PROCEDURE #34

Inactive Protocol Animal Holding

A. General

The possibility exists that a protocol can become inactive (e.g., protocol approval expires due to lack of timely annual or triennial renewal, or protocol is suspended), but animals remain in an animal facility. In order to avoid euthanasia of such animals, and to remain in compliance with regulatory requirements, the Marshall University Animal Care and Use Committee (IACUC) allows transfer of such animals to the ARF Animal Holding SOP for a maximum of 90 days. During this time, the investigator of the inactive protocol must take the necessary actions to gain re-approval of the animal use protocol. Failure to gain re-approval will result in permanent forfeiture of the animals.

B. Methods

1. When animals are assigned to the Animal Holding SOP, the oversight of the animals will be the responsibility of the Attending Veterinarian or ARF veterinary staff designee.

2. During the time animals are assigned to the Animal Holding SOP, the animals will be provided routine care. Only procedures necessary to maintain the health and well-being of the animals will continue. Examples might include ongoing post-operative care, chronic catheter maintenance, administration of insulin, etc. that is performed by the research staff.

3. No experimental or teaching procedures are permitted. Such procedures include but are not limited to: data collection, sample collection, surgery, administering substances, training/performance of tasks, etc. Special diets will not be continued unless necessary for the well-being of the animals.

4. Any animal housing related charges will continue to be the responsibility of the Principal Investigator.

5. The room door and caging where the animals are housed will be identified and posted stating no research or teaching associated activities are to be conducted. Access to the animal holding room by the investigative staff will be withdrawn.

6. Upon IACUC approval of the new protocol, the animals will be transferred back to the Principal Investigator’s protocol and research or teaching activities may

resume. If the protocol is not reinstated, the disposition of the animals will be at the discretion of the ARF veterinary staff.

STANDING OPERATING PROCEDURE #35

Oral Gavage – Mouse & Rat

Oral gavage is used to dose an animal with a specified volume of material directly into its stomach.

Equipment required:

- Gavage tube with rounded tip (available through ARF)
  - Mouse: usually 20-22 gauge
  - Rat: usually 18-20 gauge
- Permanent marker
- Syringe to deliver material

Technique:

1. Only perform on an **awake** animal!

2. Measure the tube for the correct length by placing it along the outside of the animal, so that the ball tip is at the last rib and the other end of tube is by the animal's nose.

3. Mark the tube at the point where it reaches the tip of animal's nose. Do not pass the tube farther than this mark or you risk perforation of the stomach.

4. **Mouse**: Scruff mouse over the shoulders so that the skin pulls the front legs out to the side. This keeps the front feet from pushing the tube away.

Rat: Requires an assistant to hold the rat by the chest and support the lower body.

5. Hold the animal in an upright (vertical) position.

6. Extend the head back using index finger on top of the head or using the tube to raise the head so the esophagus is in a straight line.

7. Insert the tube into the right side of the animal's mouth.

8. Slide the tip gently past the back of the tongue. The gavage tube should slide down the esophagus easily, if properly placed.

9. **DO NOT FORCE!** If any resistance is met, remove the tube and reinsert.

10. Once the gavage tube is properly placed, slowly administer the material. (If animal has trouble breathing, struggles violently, or coughs, you may be in the trachea. Stop administration immediately and remove the gavage tube!)

11. After administration, slowly remove tube from esophagus.

12. Return animal to its cage.

13. Observe animal for 15-30 minutes for any signs of pain or distress **(Labored breathing, sudden lethargy, or poor mucous membrane color indicates delivery into the lungs. Any animal with these signs must be euthanized)**

Maximum Recommended Volumes*:

- **Mouse**: 0.10 ml/10 grams body weight (max. of 0.25 ml)
- **Rat**: 1.0-2.0 ml /100 grams body weight

*(Pregnant animals should only receive 25% of the maximum volume)

STANDING OPERATING PROCEDURE #36

Sentinel Animal Program

Purpose: The purpose of this SOP is to continue the sentinel animal monitoring of animals in the Marshall University Animal Resource Facility (ARF). We will place two (2) animals in each animal rack housing animals for long periods of time. At the end of six months these sentinel animals will be euthanized, have blood collected for serum analysis to determine if these animals have been exposed to a panel of diseases, and examined grossly for presence of parasites (internal and external) and signs of disease.

Aim: The primary aim is to insure we maintain a healthy research animal for the investigators in the Marshall University research community.

Methods:

Virus Antibody Free rats and mice will be purchased from an ARF approved vendor. Upon receipt two animals will be placed in a cage and placed on each rack in each animal holding room containing animals that are to be held in the ARF for long periods of time (over two months). When the animal cages are changed in each room, approximately one tablespoon of the dirty bedding from cages containing other animals will be placed in the clean cage the sentinel animals are to be placed in. These cages are usually changed once weekly. Placing the dirty bedding into the clean cage for the sentinel animals will expose the sentinels to any disease or parasitic organisms present in the animal colony. At the end of six months these sentinels will be euthanized by carbon dioxide inhalation with cervical dislocation to insure euthanasia. Blood samples will be collected by the intracardiac method and submitted to a commercial laboratory for analysis to a standard panel of diseases. The animals will be examined for the presence of external and internal parasites. A gross necropsy will be performed on each animal. If any abnormally appearing tissue is present, it will be collected, fixed, and examined microscopically for signs of disease.

STANDING OPERATING PROCEDURE #37

Recapping of Needles

Purpose: To ensure that all employees using needles are familiar with proper handling techniques.

Reference: Occupational Health and Safety Administration regulation 29 CFR 1910 §1030(d)(2)(vii)(B)

Methods:

A. 29 CFR 1910 prohibits recapping of contaminated needles unless recapping is required by a specific medical procedure or unless no alternative is feasible. If recapping must be performed, it must be accomplished by means of a recapping device which adequately protects the hands or a properly performed one hand scoop technique.

B. Needles are not to be recapped. Instead, syringes with attached uncapped needles are to be dropped into puncture proof containers for disposal. Such containers must be located in every room in which sharps are used. ***NOTE:*** *In some instances, neutralization of a hazardous agent prior to syringe and needle disposal may be necessary*.