



# VA Pittsburgh Healthcare System Animal Research Facility: Standard Operating Procedures

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## **Part I: Introduction**

## **Standard Operating Procedures for Animal Research Facility Operated by the VA Pittsburgh Healthcare System**

This compilation of Veterans Affairs (VA) Medical Center Animal Research Facility (ARF) Standard Operating Procedures (SOP) is a reference for investigators, Institutional Animal Care and Use Committee (IACUC) members, and ARF staff. These SOPs detail the policies and procedures related to the care and use of laboratory animals within the VA Pittsburgh Healthcare System (VAPHS) ARF.

### **Ethical Principles Governing the use of Animals in Research**

Animal subjects contribute immeasurably to advancements in medical science. Most research and testing involving human patients is based on the results of animal experimentation. To provide hope for veterans suffering from diseases that currently lack cures or effective treatments, the VA actively supports the use of animals in research, teaching, and testing. However, the use of animals in VA research is a privilege granted with the understanding and expectation that such research is conducted according to the highest ethical and legal standards.

### **The Regulatory Mandates for Animal Experimentation**

All animal care, husbandry, and animal research practices at VA animal facilities must be in accordance with applicable laws, regulations, and policies. The basic principles governing animal research in VA are found in the United States (U.S.) Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, which include the following imperatives:

- Animal experiments are undertaken only after due consideration of their relevance for human or animal health and the advancement of biological knowledge.
- The fewest number of animals needed to achieve scientific objectives is to be used.
- The least sentient species that will permit the attainment of research objectives is to be used.
- The least painful or distressful procedures needed to meet research objectives are to be used, and all reasonable measures to minimize pain and distress should be utilized.
- When planning and conducting studies, the principles of replacement, reduction, and refinement need to always be considered.
- Procedures that would be considered painful in a human should be considered to be painful in animals.

- The best possible living conditions need to be maintained for animals kept for research, training, or testing purposes. Animal care needs to be supervised by a veterinarian experienced in laboratory animal medicine. Housing needs to ensure that the general health of animals is safeguarded and that undue stress is avoided, with appropriate attention paid to environmental factors such as temperature, ventilation, and humidity.
- Personnel need to have appropriate qualifications, training, and experience when conducting procedures on animals. Opportunities for hands-on training must be provided as needed.

All animal research must comply with the Health Research Extension Act (codified at 42 U.S.C. Section 289d) and the Public Health Services (PHS) Policy. The PHS Policy includes the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (prepared by the Interagency Research Animal Committee), The Guide for the Care and Use of Laboratory Animals (prepared by the National Research Council), henceforth called the Guide, and the Report of the AVMA Guidelines on Euthanasia. NOTE: Compliance with PHS Policy is mandated by VA policy, whether or not PHS funds are accepted by an individual VA facility. All animal research must be covered by a PHS Assurance. By law, all animal research must comply with the Animal Welfare Act [codified at 7 U.S.C. Sections 2131-2159, the USDA AWAR (Animal Welfare Act Regulations and Standards), Title 9 Code of Federal Regulations (CFR) Parts 1-4, and 42 CFR 73, Possession, Use, and Transfer of Select Agents and Toxins]. All VA animal research involving infectious or recombinant agents must also comply with guidelines found in the latest editions of the Centers for Disease Control and Prevention (CDC)-National Institutes of Health (NIH) publication entitled "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) and the NIH publication entitled "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" (NIH Guidelines).

Any research conducted within the VAPHS ARF must be reviewed and approved by the VAPHS IACUC and notification issued by the VAPHS Associate Chief of Staff for Research and Development (ACOS/R&D) before the project can begin. Please refer to the VAPHS IACUC SOPs for additional information regarding this process (Appendix A).

## **Definition of Animal Subject and Research**

Animal research refers to any use of laboratory animals in research, testing, or training. The term "**animal**" is defined as any live vertebrate animal used or intended for use in research, research training, experimentation, biological testing, or for a related purpose (see PHS Policy on Humane Care and Use of Animals, Sec. III). For the purpose of compliance with the Animal Welfare Act Regulations an animal is defined as any live or dead cat or dog, non-human primate, guinea pig, hamster, rabbit, or any other warm-blooded animal that is being used, or is intended for use in research, teaching, testing, or experimentation. The term excludes birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, horses not used for research purposes and other farm animals, such as, but not limited to livestock or poultry, used or intended for use as food or fiber, livestock or poultry used or intended for use in improving animal nutrition, breeding, management, production efficiency, or for improving the quality of food or fiber.

## **Part II: Maintenance and Sanitation Procedures**

## A. General Maintenance and Sanitation Requirements

1. Mop buckets - After each use, buckets are to be washed out with a mixture of hot water and a disinfectant, and then rinsed with hot water. Once a week the mop buckets and ringers are sanitized by hand with Quatricide or equivalent disinfectant, and rinsed with hot water.
2. Mops – A clean mop head must be used for each animal, procedure and operating room and must be washed and dried using a washing machine and dryer after use.
3. Brooms and Dustpans – In order to prevent contamination, each room must have its own broom and dustpan.
4. Work Clothing and Shoes – All ARF Staff are required to wear scrubs while working in the ARF. Prior to leaving the building, personnel are required to change into street apparel. The scrubs are to be laundered (using both the washer and dryer) in the ARF. Personnel must have a separate pair of shoes specific for work within the ARF.
5. Bedding – No bedding is to be accepted in bags that are or have been wet, broken or torn. Sawdust is unacceptable as bedding for the animals.
6. Feed – Upon arrival, all bags of feed are to be inspected immediately for milling dates. These dates must be within 30 days of delivery. The feed must be used within the time-frame specified below:

Rat/Mouse/Rabbit– Must be used within 180 days of the milling date.

Feed in bags, which are or have been wet, broken or torn cannot be accepted or used.

7. Soiled bedding removal – All soiled cages and cage pans are to be removed from the animal housing rooms and transferred to the dirty side of the cage washing area. The bedding is to be removed (scraped and dumped) and the cages and pans are to be sanitized in the cage washer. Soiled bedding is not to be removed and replaced with clean bedding unless cages are sanitized first. The frequency with which bedding is changed is outlined below:
  - Shoebox cages – Once per week\* or as needed
  - Stainless steel lids – Once per month\* or as needed
  - Cage pans – Three times per week\*
  - Cage racks and shelves – Once per month\* or as needed

\*Frequency may increase depending on the condition of the animals housed in the general population (e.g., diabetic, breeding) and for animals in quarantine.

Cages used to house animals in quarantine are scraped in the animal housing room, sprayed with a disinfectant, rinsed and transported to the dirty side for placement in the cage washer.

## **B. Area-Specific Cleaning Procedures**

Cleaning and sanitation records are kept for all rooms.

### **1. Closets GA126 and GA129:**

- Wipe off shelves once a week or more if needed. Everything on shelves is to be kept neatly folded.
- Plastic bags are to be kept neatly folded.
- Scour the sink in room 126 once a week.
- Sweep floors and mop once a week or more frequently, if needed.

### **2. Dead Animal Freezers and Disposal:**

- Keep freezers clean inside and out at all times.
- Seal animal carcasses and tissues in leak proof plastic bags, which are located next to the freezer in the middle hallway. They should be bagged and placed in the freezer by the Principal Investigator (PI), their staff, or ARF Staff and kept in the freezer until an approximate weight of 22 pounds is met. They are then placed in a red biohazard waste bag by the ARF Staff, and then placed in biohazard boxes with the weight of the boxes not to exceed 50 pounds.

### **3. Stair Cases:**

- Sweep and mop staircases once a month or more frequently, if needed.
- Wipe hand railings with hot water and a disinfectant once a month.

### **4. Hallways:**

- Sweep floors and mop every day or as needed.
- Sanitize walls and ceiling every three to six months.
- Dust accessories mounted on walls once a week and sanitize.
- Dust overhead vents weekly and clean with walls.

## **5. Employee Break Room:**

- Keep room clean at all times.
- Table, microwave, refrigerator, and cabinet must be free of food and drink spills.
- Sweep and mop floor once a week or more frequently if needed.
- Wipe air vents, door and doorframe once a week.

## **6. Ladies Locker Room 1A107:**

The ladies locker room should be kept clean at all times. The following procedures should be conducted as necessary using Quatricide, Hypochlor, or other equivalent disinfectant.

- Wipe toilet, sinks, and counter- top
- Wipe mirrors
- Wipe shower stall and toilet stall
- Wipe lockers inside and out
- Empty trash containers
- Fill paper towel dispenser
- Wipe air vents, door, and doorframe
- Sweep and mop floor

## **7. Surgery Area:**

Areas are checked daily for cleanliness. If an area has been used, perform the following:

### **A. Pre-op Room:**

- Dispose of full sharps containers and replace with new sharps containers
- Empty biohazardous waste bags
- Clean countertops and equipment with Quatricide or equivalent disinfectant
- Sweep floors and mop with Quatricide, Hypochlor or equivalent disinfectant
- Wipe off doors with Quatricide or equivalent disinfectant

- Check surgical supplies and replace/order as needed
- Sanitize areas every three months including ceilings and attachments

## **B. Operating Rooms and Scrub Area**

- Before surgery, check all equipment to ensure proper working condition.
- After Surgery:
  - Clean all flat surfaces with Quatricide or equivalent disinfectant.
  - Dispose of full sharps containers and replace with new containers.
  - Remove biohazardous waste bags and box them.
  - Clean all moveable equipment with Quatricide or equivalent disinfectant and remove from the room.
  - Sweep floors and then mop with Quatricide, Hypochlor or equivalent disinfectant.
  - Clean doors with Quatricide or equivalent disinfectant.
  - Scour scrub sink with cleanser then polish with stainless steel polish.
  - Sanitize rooms every three months using Quatricide or equivalent disinfectant.

## **8. Procedure Room, Autoclave Room, and Necropsy Room**

- Check rooms daily for usage.
- If room has been used:
  - Dispose of full sharps containers and replace with new containers.
  - Remove biohazardous bags and box the waste. Replace the biohazardous bags with new bags.
  - Clean counter tops and equipment with Quatricide or equivalent disinfectant.
  - Sweep and mop floors.
- Wipe doors with Quatricide or equivalent disinfectant.
- Sanitize room every three to six months with Quatricide or equivalent disinfectant.

## 9. Cage Washer Areas\*:

### A. Clean Side:

- Sweep floors and mop weekly or as needed.
- Place all equipment neatly on shelves.
- Wipe all shelves as needed and sanitize every 3 months.
- Sanitize all utility carts daily.
- Sanitize room every three months with Quatricide or equivalent disinfectant.
- Polish stainless steel on cage washer with stainless steel polish as needed.
- Check floor drain and clean daily.
- Clean door and window as needed with glass cleaner.

### B. Dirty Side

- Sweep floors and mop daily or as needed for all spills.
- Clean all equipment as used and replace neatly.
- Empty rubbish barrels and clean as needed and sanitize daily.
- Sanitize room every three months using Quatricide or equivalent disinfectant.
- Polish stainless steel cage washer and sink (inside and out) daily using stainless steel polish and clean and polish as needed.
- Check floor drain and clean (swept and mopped) daily.
- Clean doors weekly with Butcher's Disinfectant Spray, Quatricide, Hypochlor or equivalent disinfectant.
- Check the cage washer and clean weekly, if necessary. Clean the cage washer doors and polish with stainless steel polish.

**\*Safety glasses and/or face shield and hearing protection are available for use by ARF Staff.**

## **C. Laundry at the VAPHS ARF**

### **1. Mop heads and rags**

1. Set dials on washing machine to high water level and hot water setting.
2. Use one-half cup to three-fourth's cup of Tide with Clorox detergent or equivalent detergent.
3. Allow water to fill half way before adding mops and rags.
4. After completion of all cycles and machine turns off, place mops and rags in dryer for fifty to sixty minutes, depending on size of load.

### **2. Scrubs, reusable lab coats and wraps (drapes)**

1. Set dials on washing machine to high water level and hot water settings.
2. Use one-half cup to three-fourth's cup of Tide with Clorox detergent or equivalent detergent.
3. Allow water to fill half way before adding clothes and wraps.
4. After completion of all cycles and machine turns off, place clothes in dryer for thirty to fifty minutes depending on size of load.

## **D. Cleaning and Maintenance of Equipment**

### **1. Disinfection of Animal Transport Cartons**

Animals are delivered to the receiving area at the end of the corridor (between rooms GA101 and GA140). They are taken into the cage wash room (GA101) where the cartons are wiped with a disinfectant. The cartons are then transported to the designated room where the animals will be housed.

### **2. Cleaning the Cage washer**

#### **Preventative Maintenance:**

- Daily – Prior to the initial start-up of the washer, inspect the interior of the wash compartment. Keep in mind that some parts of the washer may still be very warm from prior use. Inspect all jets and remove any debris from them by poking a small wire into the jet orifice. If debris is considerable, remove pipe plug located on header manifold and run pump manually to flush out jets. Remove any debris or broken glass found on or under the pump screen. Check around the door seals and remove any debris, if found.

- Weekly – When the washer is not in operation and is cool, clean water spots and stains from the exterior of the cabinet with a stainless steel cleaner. WD-40 or similar penetrating oil works very well for this. Check all water, air and steam fittings for signs of leakage and repair as required. Run an acid cycle to de-scale the cage washer.
- Quarterly – A service contract provides service and quarterly maintenance inspections of the cage washer. Due to various fuses, seals and other repairable items, only a trained professional should perform any of these procedures. A detailed checklist and service maintenance report will be submitted after every inspection and delivered to the ARF Supervisor.

### 3. Changing Shoebox Type Caging

1. Take a cart with clean bedding-filled boxes, wire lids, filter lids (if needed), a bucket of mild disinfectant in warm water, a clean rag, and exam gloves into the animal room.
2. Take one box off of the shelf at a time and place on your cart.
3. Wipe the shelf area where the dirty box was located with your disinfectant rag.
4. Transfer the animals from the dirty to clean cage, fill the feeder, put on the clean water bottle, and return the cage to the shelf. Dip gloved hands into diluted disinfectant between each cage change.
5. Remove a small amount of dirty bedding, to include some feces, from each dirty cage and place this material into an empty (no bedding material) clean cage; this cage will be used for the sentinel animals included in the mouse and rat disease surveillance program (Section IV).
6. Repeat this procedure until all cage changes are completed.
7. Cage card holders, lids and bonnets must be changed once a month or more if necessary.
8. Wipe tops of shelves, wheels, lights and doors with disinfectant cloth.
9. Stack the dirty cages and lids on a cart and take them to the dirty side of the cage washer room for scraping.
10. All procedures are subject to change according to the needs of the facility.

## **Part III. Animal Care**

## **Animal Care**

### **Rats and Non-Immunocompromised Mice**

1. First thing every day, check cages to note the condition of the animals. An ARF Staff member will check the animal cages daily for visible signs of change or distress, such as leaky bottles, birth of new pups, decrease in food or water consumption, blood in cage, wounds, secretions around the eyes, nose and genital area, respiratory distress, constipation, diarrhea, swelling, sluggishness, gait, dull coat or loss of hair. All concerns will be reported to the ARF Supervisor and depending on the severity of the concern, the PI/technician and/or attending veterinarian will be notified.
2. Change cages at least once per week or more often as needed. During cage changes, animals are inspected for any abnormal conditions as listed above in section 1. Dirty bedding is removed on the dirty side of the cage washer.
3. Check the water bottles every day and add fresh water as needed. Check all cages for 'DO NOT WATER" labels. Be sure to return each bottle to its original cage.
4. Check the feed every day and refill if necessary. Check all cages for "DO NOT FEED" labels.
5. Sanitize the water bottles once a week.
6. Change shelves, cage card holders, lids and bonnets once a month
7. Sanitize room every three to six months.
8. Sweep the floor and mop weekly or as needed.
9. Wipe the feed barrel weekly.
10. Ensure that the plastic bags in the feed barrels are in good condition. No loose feed is to be kept in barrel.
11. Wipe off air vent weekly.
12. Each cage must have an identification card with the following information: protocol number, investigator's name, date received, strain, sex, date of birth, and number of animals per cage.
13. Only store items that are essential to the animal care in the housing room.
14. Check the floor drains every day and flush out if necessary.
15. Wipe the doors weekly.
16. Document the inventory on Monday, Wednesday, and Friday or more often if needed.

## **Animal Care Nude and Severe Combined Immunodeficiency (SCID) Mice**

1. Upon entering the housing room of immunocompromised animals, all individuals must wear the following personal protective equipment (PPE): gown, surgical mask, shoe covers and gloves. Sterile gloves are required for opening a micro-isolator unit to handle the animals.
2. Change the cages and lids and sanitize once a week or more often if needed. Remove bedding on the dirty side of cage washer.
3. Check the feed every day and refill when necessary. Check all cages for "DO NOT FEED" and "DO NOT WATER" labels.
4. Check the water bottles every day. If necessary a new bottle of sterile water is given.
5. Wipe the shelves off weekly or as needed.
6. Sweep the floors and mop weekly or as needed.
7. Wipe the air vents off weekly or as needed.
8. Wipe off the doors weekly or as needed.
9. Each cage must have an identification card with the following information:  
protocol number, investigator's name, date received, strain, sex, date of birth, and number of animals per cage.
10. Sanitize the shelves monthly or more often if needed.
11. Sanitize the room every three months
12. Enter the dates that the rooms and cage shelves are sanitized into the room's logbook.
13. Document the inventory on Monday, Wednesday, and Friday, or more often if needed.
14. Autoclave all caging, food, water bottles and bedding.

## Cage Size and Weight Limits For Rats

Space allocations for a 19 inches x 10.5 inches x 8 inches (199 square inches [sq.in.] floor) polycarbonate shoebox cage, as specified in the Guide for the Care and Use of Laboratory Animals 8<sup>th</sup> Edition are as follows:

Weight (grams-g)	Floor area/animal (sq. in.)	Height cage floor to cage top (in)
Under 100	17	7
Up to 200	23	7
Up to 300	29	7
Up to 400	40	7
Up to 500	60	7
Over 500	70 or more	7

The following number of rats per cage is allowed based on the following weights:

- 1 rat over 500 g
- 2 rats up to 500 g each
- 3 rats up to 400 g each \*
- 4 rats up to 300 g each \*
- 5 rats up to 200 g each \*

As social species, the standard of housing for rats and mice (see below) are in pairs or groups rather than individually. Unless approved for scientific reasons by the IACUC, there are no exceptions to the minimum space or social housing requirements. The attending veterinarian or designee may exempt individual animals from social housing on a per case basis due to social incompatibility or due to other health or welfare concerns. Additional cage enrichment is required for animals singly housed due to either medical or protocol dispensation.

\*The VAPHS ARF recommends no more than 2 rats at 250 g each per cage. This does not apply to mothers with pups or to weanlings under 100 g each.

Rats have an acclimation period of 48 hours for all procedures performed unless specified in an approved IACUC protocol. Use of rats during the 48 hour acclimation period is allowed for IACUC approved non-survival surgeries, tissue harvest, and breeding.

## Cage Size and Weight Limits

### For Mice

Required cage floor space and height as specified in the Guide for the Care and Use of Laboratory Animals 8<sup>th</sup> edition are as follows:

Weight (g)	Floor area/animal (sq. in.)	Height cage floor to cage top (in)
Under 10	6	5
Up to 15	8	5
Up to 25	12	5
Over 25*	15 or more	5

For an 11.5 inches x 7.5 inches x 5 inches (70 sq. in. floor) polycarbonate cage, the number of mice allowed is based on the weights listed:

- 4 mice over 25 g
- 5 mice up to 25 g each \*
- 7 mice up to 15 g each\*
- 10 mice up to 10 g each\*

For an 18.5 inches x 10 inches x 6 inches (185 sq. in. floor) polycarbonate cage, the number of mice allowed is based on the weights listed:

- 8 mice over 25 g
- 10 mice up to 25 g each
- 14 mice up to 15 g each
- 20 mice up to 10 g each

\*The VAPHS ARF recommends no more than 4 mice at any weight in the small mouse cage. This does not apply to mothers with pups. Only one litter is allowed per cage.

Mice have an acclimation period of 48 hours for all procedures performed unless specified in an approved IACUC protocol. Use of mice during the 48 hour acclimation period is allowed for IACUC approved non-survival surgeries, tissue harvest, and breeding.

## **Animal Care Rabbits**

1. First thing every day, check cages to note the condition of the animals, such as deaths, caught feet, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total loss of water and/or food consumption. Report any of these problems immediately to the ARF Supervisor and/or PI.
2. Change the bedding in the pan on Monday, Wednesday and Friday. Pans are removed and replaced with sanitized pans (soiled bedding is removed on the dirty side of cage washer room). Pans for rabbit cages must be free of urine scale.
3. Check the water bottles every day and add fresh water as needed. Check all cages for 'DO NOT WATER" labels. Be sure to return each bottle to its original cage.
4. Check the feed every day and refill if necessary. Check all cages for "DO NOT FEED" labels.
5. Sanitize the water bottles once a week or more often if needed.
6. Sanitize the rabbit cages every two weeks or more often if needed (no dirty empty cages should be stored in the animal room).
7. Sweep the floor and mop weekly or as needed.
8. Wipe the feed barrel weekly or as needed. The plastic bags in the feed barrels are to be kept in good condition and no loose feed is to be kept in barrels.
9. Wipe the doors, including the vision panel weekly or as needed.
10. Wipe the air vent weekly or as needed.
11. Once a week, check the rabbits for long incisor teeth and long nails. Trim over grown incisors and nails as needed.
12. Each rabbit must have an identification card on its cage with the following information: assigned identification number, protocol number, investigator's name, sex, date received, and number of animals per cage.
13. Document the inventory on Monday, Wednesday, and Friday or more often if needed.
14. The dates that the rooms and cage shelves are sanitized must be entered into the room log book.
15. Nothing is to be placed on top of the cages.
16. Check the floor drains every day and flush with water if necessary.

## **Cage Size and Weight Limits For Rabbits**

1. Lab Products Rabbit Unit with 8 cages

<u>Cage Size</u>	<u>Allowable Rabbit Weight (pounds-lbs.)</u>
With divider - 4 sq. ft.	1 rabbit up to 11 lbs.
Without divider - 8 sq. ft.	1 rabbit over 11 lbs.

2. Allentown Rabbit Unit with 6 cages

<u>Cage Size</u>	<u>Allowable Rabbit Weight</u>
With divider - 4 sq. ft.	1 rabbit up to 11 lbs.
Without divider - 8 sq. ft.	1 rabbit over 11 lbs.

**Information obtained from the Guide for the Care and Use of Laboratory Animals 8th edition-National Academy Press 2011**

Rabbits have an acclimation period of 7 days.

## **Part IV: Mouse Quarantine**

Mice from documented specific pathogen-free approved commercial sources do not undergo quarantine upon entry. These animals have an acclimation period of 48 hours for all procedures performed unless specified in their approved IACUC protocol.

All mice entering the VAPHS ARF from sources other than standard approved commercial vendors will be quarantined. When used in this SOP, the term “imported mice” refers to mice that come from facilities other than the approved commercial suppliers. Mice available commercially through approved vendor sources may not be imported through unapproved sources, collaborative or not.

### **Arranging for mice shipment:**

After mice have been approved to be imported (see VAPHS Mouse Import Requirement below), the importing investigator must contact the ARF Supervisor to arrange for space and to set a time for bringing the mice into the facility.

## **1. Mouse Importation, Housing, and Husbandry**

### **Housing of Imported Mice:**

Upon subsequent approval and receipt, the animals are housed in quarantine for six weeks under close observation. During that period, imported mice are housed in individually vented cages in a dedicated quarantine room. Imported mice will be housed in a ventilated cage rack operated in the negative air flow mode. The ventilated rack is stored in a room near the dirty side of the cage washer. The air pressure in the quarantine room is negative to the corridor. Breeding of mice may be done while the mice are in quarantine but only if absolutely necessary to preserve the strain. Other research manipulations may be conducted during quarantine only with the expressed consent of the IACUC.

### **Entering and Leaving a Quarantine Room:**

Only necessary ARF Staff and necessary investigative staff will be permitted in quarantine rooms. Other mouse rooms are not to be re-entered on the same day after the quarantine room has been accessed (i.e. the quarantine room is a “do last” area). Before entering the quarantine room, individuals are to don fresh shoe covers (second pair), gown or laboratory coat, and gloves. These items are to be removed just before leaving the quarantine room and placed in a receptacle provided for these items.

### **Caging and Cage Changing: Laminar Flow Units**

Imported mice will be housed in laminar flow, individually vented micro-isolation cages. These cages will be autoclaved only when known immuno-incompetent mice are imported. Only one dirty cage of mice may be open at any time. An empty clean cage will be open to receive dirty bedding when sentinel mice are to be exposed to dirty bedding. Operators will dip their gloved hands in disinfectant solution before removing the filter cap from each cage. The work surface for cage changes will be wiped with a disinfectant before each cage is placed on it. Clean and dirty cages are to be stored on the work surface of the laminar flow unit. Dirty cages are to be scraped under the laminar flow hood, sprayed with a disinfectant and then washed in the quarantine room after a 5-10 minute exposure to the disinfectant. Bedding is double bagged (biohazardous) in the housing room and placed in a biohazard box located outside of the quarantine room. Bonnets and food/water trays are

to be sprayed with a disinfectant once a week and all items are to be transferred to the dirty side and placed on a cage rack to go through the cage washer. The wheels of the cart used to transport the dirty cages to the cage washer are to be sprayed with a disinfectant as it is rolled from the quarantine room.

Individuals moving cages from the quarantine room are to remove the PPE that they donned prior to entering the room (see Entering and Leaving a Quarantine Room directly above).

#### **Disinfection of Quarantine Rooms:**

All surfaces of the quarantine room(s) will be disinfected weekly with Hypochlor or equivalent disinfectant. The normal sanitations schedule for the facility will apply to quarantine rooms; this includes weekly mopping of the rooms with Hypochlor or equivalent disinfectant (mops in the quarantine rooms will not be used elsewhere in the facility) and mopping of the corridors with Hypochlor or equivalent disinfectant late in the day.

#### **Testing of Imported Mice for Pathogens:**

The mice will be sent to an appropriate commercial laboratory and comprehensively tested for murine pathogens as outlined below (see Part VI: Mouse and Rat Disease Surveillance Program). The presence of pathogens will be assessed in the imported mice by housing pathogen-free sentinel mice in bedding collected from all cages of imported mice every time the cages are changed and/or cleaned. Dirty bedding is placed in an empty clean cage (the more dirty bedding and feces the better). Two sentinel mice are to be housed for every 20 cages of imported mice. The sentinel mice are tested after being exposed to dirty bedding for at least 4-6 weeks.

#### **Release of Mice from Quarantine:**

Mice will be released from quarantine by the Veterinarian if the tested mice are free of VA excluded mouse pathogens. The ARF Supervisor will communicate with the investigator to arrange for the imported mice to be moved to an animal housing room in the ARF.

Variations: Removal of animals from the quarantine room for acute terminal use in investigative laboratories during the active quarantine period can be considered on a case by case basis if an appropriate rationale is provided to the IACUC. If granted, this must be preceded by a comprehensive strategy meeting to review transportation, containment and subsequent trafficking constraints.

#### **Handling of Mice that cannot be Released from Quarantine:**

Imported mice that are shown to have a pathogen or pathogens that are not already in the VAPHS ARF mice will not be released from quarantine. If any pathogen is detected, the testing results will be validated and a course of action (treatment, eradication, re-derivation, etc.) will be made based on the agent(s) in question. Any such options dealing with confirmed excluded pathogens will be discussed and approved by the IACUC before implementation.

## **2. Mouse Importation Requirements**

The following items are required for every rodent import request:

### Shipping Application:

To initiate the import process, the receiving investigator is required to submit an Animal Import/Export Application form to the ARF Supervisor completing all information requested therein.

### Health and Facility Status:

The health requirements for importation of animals into the VAPHS ARF are that the sending facility<sup>1</sup> in its entirety must be free of all pathogens that are currently excluded from our rodent colonies for at least the previous nine months. This must be documented by no less than three negative (quarterly) sentinel health test reports in that time period. The most recent of these results should have been performed within 30 days of export. If these requirements cannot be met, then direct importation of rodents will not be allowed and alternate methods of animal procurement (e.g. embryo transfer or re-derivation) will be necessary.

In addition to the required nine months of negative sentinel health reports specified above, the health status of the entire facility from where the rodents are to be exported should be provided (again through serial sentinel health reports) for at least 18 months prior to export. Details concerning how any infectious processes that may have occurred during the period between months 10-18 should be described along with how these diseases were mitigated. If the animals in question have been relocated during this time period of 18 months, the health history should include both the current and previous housing locations.

### Excluded pathogens at this institution are as follows:

**Mice:** SEND (Sendai virus), PVM (Pneumonia virus of mice), MHV (Mouse hepatitis virus), MVM (Minute virus of mice), MPV (Mouse parvovirus), TMEV (Mouse polio virus), REO (Reovirus), MPUL (*Mycoplasma pulmonis*), EDIM (Epizootic Diarrhea of Infant Mice), LCMV (Lymphocytic Choriomeningitis Virus), MAV (Mouse adenovirus FL/K87), ECTRO (Ectromelia virus), K (Mouse pneumonitis virus), POLY (Polyoma virus), MTLV (Mouse Thymic virus), MCMV (Mouse cytomegalovirus), HANT (Hantaan virus), ECUN (*Encephalitozoon cuniculi*), CARB (Cilia-associated respiratory bacillus), pinworms of the Genera *Aspicularis* and *Syphacia*, and ectoparasites.

Helicobacter infection is not necessarily an exclusionary factor for importation (depending on the nature of the research study in question and the direct concerns of the PI doing the importing), however; depending on the nature of the research in question, it may be appropriate to determine the infection status of the animals involved and/or implement treatment prior to shipment. In addition, mice that test positive for murine norovirus (MNV) are not excluded from importation. The status of these animals must also be determined and reported prior to shipment.

**Rats:** SEND, PVM, RCV/SDAV (Rat coronavirus/Sialodacryoadenitis), RV (Kilham rat virus), H-1 (Toolan's H-1 virus), TMEV, REO, MPUL, LCMV, HANT, MAV, ECUN, CARB, RPV (Rat parvovirus), REV (Rat Enterovirus), pinworms of the Genera *Aspicularis* and *Syphacia*, and ectoparasites.

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<sup>1</sup> A facility is loosely defined as a collective set of rooms, corridors or floors housing animals integral to a specific building. The term is subject to considerable professional judgment that may either expand or restrict this definition, factoring in matters such as the shared use of cage wash systems, procedural space and animal husbandry personnel as well as general trafficking patterns and other criteria.

**Animal Identification:**

All animals must have a means of permanent identification, such as: ear tag, ear notch, toe clip, tattoo, or microchip. An ID code must be included with the shipment.

**Strain:**

The strain name must be given in its entirety (for example, C57BL/6-Tg (ACTbEGFP) 10sb/J should not be abbreviated to C57BL) to prevent identification errors.

**Breeding Pairs and Male Rodents:**

Males and females sent as breeding pairs must be shipped in separate shipping boxes or shipping box compartments to prevent breeding in transit. With the exception of litter mates, males should be shipped separately to prevent fighting during shipment.

Please contact the ARF Supervisor with any questions regarding the above policies.

## **Part V. Breeding Rodents**

## **Animal Breeding:**

White identification cards are required for each rodent used for breeding. Along with the identification card, each female that is used for breeding, must also have a Breeding card. These cards shall follow the mouse from cage to cage. Info that must be included on the ID cards:

1. Investigator's name
2. Protocol number
3. Date received
4. Gender
5. Strain
6. Date of birth
7. Number of animals/inventory

On the Breeding Card, the PI/research staff must indicate the date that males and females are put into the same cage for breeding. Additionally, the male's and female's identification number must be on the card so that good and bad breeders can be tracked.

If the PI or their staff has not already done so, the ARF Staff will write in the date they notice that the female is pregnant on the breeding card.

It is the responsibility of the PI/research staff member to know when pups are due and to be there to confirm the date of birth, if necessary. If the ARF Staff discover the pups in the box and the PI/research staff member has not been in to confirm the date of birth, the ARF Staff will enter the date that the pups were found as the date of birth (minus one to seven days depending on when the box was changed last) on the Breeding Card. Information on the Breeding Card provides the technician and ARF Staff with a record of the number of pregnancies, the length of the pregnancy, the number of live births versus the number of still births, whether the female kills her pups, etc. The ARF Staff will monitor the females closely so as to prevent any undo pain or suffering for the mother or her pups.

Finally, the PI/research staff member will mark the date that the pups are to be weaned from their mothers, (pups may be left with the mother longer if small but the wean date will be the date the pups are added to the investigator's inventory).

## **Part VI: Mouse and Rat Disease Surveillance Program**

The following procedures are to be followed to assess for disease in mice and rats.

1. Purchase two 3-4 week old non-inbred animals (mice or rats as applies) from an approved vendor each time sentinels are sent for testing.
2. House at least two sentinel animals in active housing rooms. The attending veterinarian will determine which rooms require sentinel animals and how many are required.
3. During cage changing, place a small amount of dirty bedding from each monitored cage into a clean empty cage that will house the sentinel animals.
4. After the cages on the rack have been changed, place the sentinel animals in the new cage that contains dirty bedding. A filter cap is placed on the sentinel cage.
5. Every four months submit one animal from each housing room for testing. The second sentinel animal is to remain in the cage until quarterly results from diagnostic labs are received. After getting approval from the attending veterinarian, introduce the old sentinel to the new sentinels by housing them together for a period of one month. The older sentinel will be removed and euthanized. The two new sentinel animals are exposed to the old sentinel in case any pathogen was incubated when animals were submitted for testing.
6. Sentinels are tested for standard rodent pathogens, internal and external parasites, and bacterial pathogens.

**Excluded pathogens at the VAPHS ARF are as follows:**

**Mice:** SEND (Sendai virus), PVM (Pneumonia virus of mice), MHV (Mouse hepatitis virus), MVM (Minute virus of mice), MPV (Mouse parvovirus), TMEV (Mouse polio virus), REO (Reovirus), MPUL (*Mycoplasma pulmonis*), EDIM (Epizootic Diarrhea of Infant Mice), LCMV (Lymphocytic Choriomeningitis Virus), MAV (Mouse adenovirus FL/K87), ECTRO (Ectromelia virus), K (Mouse pneumonitis virus), POLY (Polyoma virus), MTLV (Mouse Thymic virus), MCMV (Mouse cytomegalovirus), HANT (Hantaan virus), ECUN (*Encephalitozoon cuniculi*), CARB (Cilia-associated respiratory bacillus), pinworms of the Genera *Aspiculuris* and *Syphacia*, and ectoparasites.

**Rats:** SEND, PVM, RCV/SDAV (Rat coronavirus/Sialodacryoadenitis), RV (Kilham rat virus), H-1 (Toolan's H-1 virus), TMEV, REO, MPUL, LCMV, HANT, MAV, ECUN, CARB, RPV (Rat parvovirus), REV (Rat Enterovirus), pinworms of the Genera *Aspiculuris* and *Syphacia*, and ectoparasites.

7. Otherwise, sentinel cages are handled like the cages for the normal population described in the cage changing procedure for non-immunocompromised rodents (see Animal Care: Rats and Non-immunocompromised Mice).

## **Part VII: Anesthesia, Analgesia, and Euthanasia**

It should always be considered that responses to drugs vary greatly within each species depending upon factors such as sex, age, weight, time of day (due to circadian rhythms), interaction with other drugs, exposure of animals to various environmental chemicals, etc. Therefore, care should always be taken to give drugs cautiously and to the desired effect before proceeding with any procedure which might cause pain.

In selecting an anesthetic for experimental animals, consideration must be given to requirements such as: 1) anesthetic duration; 2) recovery time; 3) degree of analgesia and muscular relaxation; 4) availability of equipment and personnel; and 5) the pharmacodynamics of the drugs on the organ system being studied.

Combining anesthetic agents in laboratory animals may have distinct advantages over the use of single agents by facilitating restraint or induction, improving muscular relaxation, increasing analgesics and easing recovery. However, the use of more than one drug makes accurate evaluation of the pharmacodynamic effects of the anesthetic combination more difficult and may necessarily be avoided for some studies.

**Definitions and General Information:** Glossary terms from American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2013 edition.

Acceptable: A method considered to reliably meet the requirements of euthanasia.

Acceptable with Conditions: A method considered to reliably meet the requirements of euthanasia when specified conditions are met.

Adjunctive methods: A method of assuring death that may be used after an animal has been made unconscious.

Anesthesia General: A method to produce unconsciousness.

Distress: The effect of stimuli that initiates adaptive responses that is not beneficial to the animal – responses to stimuli that interfere with its welfare and comfort.

Euthanasia: A method of killing that minimizes pain, distress, and anxiety experienced by the animal prior to the loss of consciousness, followed by cardiac or respiratory arrest and death.

Exsanguination: The action of draining an animal of blood.

Fear: An unpleasant emotional experience caused by an awareness of a threat of danger.

Pain: A sensation (perception) that results from nociception nerve impulses reaching areas of the brain capable of conscious perception via ascending neural pathways.

Secondary Method: A euthanasia method employed subsequent to a primary method to ensure death of an unconscious animal before it can recover consciousness.

Sedation: A state of Central Nervous System (CNS) depression in which the animal is awake but calm and with sufficient stimuli may be aroused.

Unacceptable: A method that does not meet the requirements of euthanasia.

Unconscious: Defined as a loss of individual awareness. An unconscious animal is recumbent, unable to perceive pain; however may respond to noxious stimulation with the spinally mediated involuntary movements depending on the degree of CNS depression present.

Analgesic agents are those, which provide relief from pain. They may be used as pre-anesthetics, in balanced anesthesia, or during post-surgical recovery periods. Opioids (e.g. morphine) and non-steroidal anti-inflammatory drugs (NSAIDs) are examples of analgesics. When systemic analgesics cannot be used, the use of local anesthetic agents, such as lidocaine, bupivacaine, etc., should be considered

Barbiturates are used primarily for the induction or maintenance of general anesthesia. They are commonly classified according to their duration of action. The effects of pentobarbital (Nembutal) can last 1-3 hours while thiamylal (Surital) and thiopental (Pentothal) provide a comparatively short amount of anesthesia, lasting only 10-45 minutes. The total amount used to produce surgical planes of anesthesia will vary depending upon the animal's condition. When administered intravenously, they should be given "to effect". Barbiturates are classified as controlled drugs.

Controlled drugs are drugs that have a potential for abuse. Some of the drugs used for animal analgesia, anesthesia, euthanasia and tranquilization that fit this category are barbiturates and narcotics. The drugs are classified into five schedules. All controlled drugs have a "C" and the schedule printed as a Roman numeral on the label. The Department of Justice Drug Enforcement Administration (DEA) requires appropriate security and record management of these substances.

Local anesthetics can be used without additional medication for minor procedures; however, restraint of the animal is still necessary. For general surgery, local anesthesia may be used in conjunction with heavy sedation and analgesia. Local anesthetics with an extended effect may provide some post-operative analgesia. Spinal anesthesia may be an alternative to general anesthesia for intra-uterine surgery in pregnant animals. Toxic levels of local anesthetics can occur, especially when used to produce epidural analgesia. The total time of anesthesia depends upon the drug, concentration, rate of injection, volume of drug injected, initial level of anesthesia, age and condition of the animal, and whether or not a vasoconstrictor is added to the solution. Some of the more commonly used local anesthetic agents are bupivacaine, lidocaine, and tetracaine. The effects of Bupivacaine (Marcaine) last significantly longer than that of any other commonly used local anesthetic. The duration can be extended with addition of epinephrine. Analgesia persists for a period after the return of sensation, thus reducing the need for strong analgesics. Injectable preparations of Lidocaine (Xylocaine) are commonly used for infiltration and nerve blocks resulting in local anesthesia. The volume injected should raise a small bubble. This preparation has a rapid rate of onset and lasts up to two hours when it contains epinephrine. Injectable preparations of Tetracaine

(Pontocaine) also may be used for infiltration and nerve blocks; however, onset of anesthesia is slower than observed with lidocaine. Tetracaine is commonly used for epidural analgesia in sheep and may be selected for surgical procedures lasting 2-3 hours.

Paralytic agents can be used in conjunction with anesthetic agents to produce more complete muscle relaxation; however, all protocols using such agents must have the prior approval of the IACUC. Investigators should personally observe any experiments in which these drugs are used and thoroughly train their personnel in appropriate use of these drugs. These agents produce a progressive paralysis which in high doses affects the respiratory muscles, thus they should only be used with extreme care. Oxygen and positive pressure ventilation should always be available when these drugs are used. These agents do not produce analgesia; therefore, no painful stimuli should be initiated without concurrent administration of analgesics or anesthetics. When paralyzing agents are used with anesthetics, it is imperative that reliable methods are available to distinguish between paralysis and anesthesia.

Narcotics are controlled drugs which provide analgesia and sedation. They can be used with other drugs for “balanced anesthesia”. Specific antagonists are available.

Tranquilizers should be used to assist in easier animal handling during induction of anesthesia, to reduce the dose of general anesthetic, and to ease the recovery from general anesthesia. Acetylpromazine (Acepromazine) is a commonly used tranquilizer in veterinary practice. Tranquilizers may be used with other drugs for “balanced anesthesia”; however, they are not analgesics.

## **Euthanasia**

Euthanasia is the act of inducing painless death. Criteria to be considered for a painless death are rapidly occurring unconsciousness and unconsciousness followed by cardiac or respiratory arrest. Although not an adequate criterion, observers may mistakenly relate any movement with consciousness and lack of movement with unconsciousness. Euthanasia techniques by which animals exhibit little or no movement are the most acceptable to most people.

Selection of the most appropriate method of euthanasia in any given situation depends upon the species involved, available means of animal control, skill of personnel, numbers of animals, economic factors, and other considerations.

The Animal Welfare Act and the National Institutes of Health (NIH) Policy state that euthanasia methods selected should be in compliance with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia.

Recommended anesthetic, analgesic, and euthanasia methods for varying species are included on the proceeding pages. These recommendations are made based upon those provided by the AVMA and Plumbs Veterinary Drug Handbook 2015, consultation with veterinary anesthesiologists, as well as experience in animal use at this institution. Additional guidance regarding anesthetic and analgesic use in rodents can be found in Appendix H.

## Mice

### 1. Tranquilizers

Agent	Dose (milligrams per kilogram)	Route	Comments
Telazol (Tiletamine HCl/Zolazepam HCL)	80 80-100	Intraperitoneal (IP) Intramuscular (IM)	For immobilization
Acetylpromazine (Acepromazine)	0.5	IM	

### 2. General Anesthetics

Agent	Dose (milligrams per kilogram)	Route	Comments
Alpha Choralose	114	IP	Scientific justification needed
Ketamine (Ketaset, Vetalar)	50-100 50	IM/IP Intravenous (IV)	Sleep produced in mice 2-3 minutes after injection of mixture; surgical anesthesia lasts about 80 minutes; full recovery about 110 minutes
Mixtures with Diazepam and Xylazine	200 mg ketamine with 5 mg Diazepam 100 mg ketamine with 5-15 mg Xylazine	IM/IP IM/IP	Sleep produced in mice 2-3 minutes after injection of mixture; surgical anesthesia lasts about 80 minutes; full recovery about 110 minutes
Xylazine (Rompun)	5 13	IM IP	Post injection; calm induction and recovery
Morphine	7-10	Subcutaneous (SC)	Give 10 mg/kg every 2-4 hours as needed
Pentobarbital (Nembutal)	40-90 40-70	IP IV	Dosage varies greatly; There is narrow margin of safety

### 3. Analgesics

Agent	Dose (milligrams per kilogram)	Route	Comments
Buprenex (buprenorphine)	0.05-0.1	SC, IM	Every 12 hours
Buprenorphine SR	0.5-1.0	SC	72 hour injection
Metacam (meloxicam)	0.2	By mouth (PO) or SC	Duration of action 24-48 hours in most species; may be used for prolonged periods of time; also effective when used in combination with opioids; use once daily
Ketaprofen	5	SC	Every 12 hours
Butorphanol	1-5	SC, IM	Every 2-4 hours
Morphine	2-5	SC	Every 2-4 hours

#### 4. Miscellaneous

Agent	Dose (milligrams per kilogram)	Route	Comments
Atropine	0.05	IM, SC	Give about 30 minutes prior to other agents

#### 5. Euthanasia\*-Injectable and Inhalant

Agent	Dose (milligrams per kilogram)	Route	Comments
Injectable - Pentobarbital	150	IP, IV	Highly effective agent when appropriately administered; retained in tissue after death
Inhalant – Carbon dioxide 70-100%	N/A	Inhalation	Safe, inexpensive and effective; tissue changes may be seen; time required to produce death may be prolonged in immature and neonatal animals#
Inhalant - Isoflurane	Induction 2-3% Maintenance 0.25-2%	Inhalation	Use a non-breathing system; nonflammable and nonexplosive; euthanasia easily performed in closed container; may produce changes in parenchymal organs

\*All deaths are to be verified; a secondary method must be performed to ensure death (e.g., pharmacologic agent, exsanguination, decapitation, or thoracotomy).

#In the case of neonatal mice, follow up with a secondary physical method (decapitation) is preferable.

#### 6. Euthanasia-Physical

Agent	Dose (milligrams per kilogram)	Route	Comments
Cervical dislocation	N/A	N/A	Acceptable with prior sedation or anesthesia; exceptions must be approved by IACUC
Decapitation	N/A	N/A	Acceptable with prior sedation or anesthesia; if unable to sedate or anesthetize because need chemical residue-free tissues, head should be immediately frozen in liquid nitrogen subsequent to severing; exceptions must be approved by IACUC
Exsanguinations	N/A	N/A	Acceptable when preceded by other methods that relieve anxiety, consciousness

## Rats

### 1. Tranquilizers

Agent	Dose (milligrams per kilogram)	Route	Comments
Telazol (Tiletamine HCl/Zolazepam HCl)	40 20-60	IP IM	For chemical restraint; for light anesthesia
Acetylpromazine (Acepromazine)	0.5	IM	
Diazepam (Valium)	3-5	IM/IP	
Ketamine (Ketaset, Vetalar)	50-100	IM/IP	

### 2. General Anesthetics

Agent	Dose (milligrams per kilogram)	Route	Comments
Alpha Choralose	55	IP	Scientific justification needed; terminal studies only
Ketamine/Xylazine (Rompun) mixture	Ketamine 40-80 mg Xylazine 5-10 mg	IM/IP	Make a mixture by adding 0.15 ml Xylazine (100 mg/ml stock solution) to each ml of ketamine (100 mg/ml stock solution); maximal anesthetic effect is achieved within 15 minutes and lasts 15-30 minutes
Mixtures with Diazepam and Ketamine	Ketamine 40-60 Diazepam 5-10	IP	Make a mixture by adding 0.15 ml Xylazine (100 mg/ml stock solution) to each ml of ketamine (100 mg/ml stock solution); maximal anesthetic effect is achieved within 15 minutes and lasts 15-30 minutes
Telazol (Tiletamine HCl, Zolazepam HCl)	20-60 40	IM IP	For chemical restraint; for light anesthesia
Thiopental (Pentothal)	20 40	IV IP	For chemical restraint
Isoflurane	Induction 2-3% Maintenance 0.25-2%	Inhalation	Use a non-breathing system; nonflammable and nonexplosive; euthanasia easily performed in a closed container; may produce changes in parenchymatous organs
Sodium pentobarbital	40-50	IP	To effect
Hypothermia	N/A	Contact	

### 3. Analgesics

Agent	Dose (milligrams per kilogram)	Route	Comments
Buprenex (buprenorphine)	0.05 0.1-0.25	IM, SC, or IV PO	Every 8-12 hours Every 8-12 hours

Agent	Dose (milligrams per kilogram)	Route	Comments
Metacam (meloxicam)	0.2	PO or SC	Once daily, has duration of action for 24-48 hours; for musculoskeletal and mild visceral pain; very effective when used in conjunction with opioids
Butorphanol	2.0	IM	Every 2-4 hours
Morphine	2.5 2.5 2.0	SC IM, SC IV	Every 2-4 hours as needed
Oxymorphone (Numorphan)	0.25-0.5	SC, IM	Duration of action 6-12 hours
Ketoprofen	5	SC	Every 12 hours

#### 4. Miscellaneous

Agent	Dose (milligrams per kilogram)	Route	Comments
Atropine	0.05	IM, SC	Give about 30 minutes prior to other agents to reduce salivary and bronchial secretions

#### 5. Euthanasia\*-Inhalant

Agent	Dose (milligrams per kilogram)	Route	Comments
Carbon dioxide 70-100%	N/A	Inhalation	Anesthesia is induced within seconds in a chamber; suitable for injection or cardiac puncture but do not try to maintain for periods in excess of 2 minutes; safe, inexpensive and effective; tissue changes associated with hypoxemia may be seen; time required to produce death may be prolonged in immature animals and neonatal animals#
Isoflurane	Induction 2-3% Maintenance 0.25-2%	Inhalation	Use a non-breathing system; nonflammable and nonexplosive; euthanasia easily performed in a closed container; may produce changes in parenchymal organs

\*All deaths are to be verified; a secondary method must be performed to ensure death (e.g., pharmacologic agent, exsanguination, decapitation, or thoracotomy).

#In the case of neonatal mice, follow up with a secondary physical method (decapitation) is preferable.

## 6. Euthanasia-Physical

Agent	Dose (milligrams per kilogram)	Route	Comments
Cervical dislocation	N/A	N/A	Suitable only in rats weighing less than 200 grams; acceptable with prior sedation or anesthesia; exceptions must be approved by IACUC
Decapitation	N/A	N/A	Acceptable with prior sedation or anesthesia; if unable to sedate or anesthetize because chemical residue-free tissues are required, head should be immediately frozen in liquid nitrogen subsequent to severing; exceptions must be approved by IACUC
Exsanguinations	N/A	N/A	Acceptable when preceded by other methods that relieve anxiety, consciousness

## Rabbits

### 1. Tranquilizers

Agent	Dose (milligrams per kilogram)	Route	Comments
Acetylpromazine (Acepromazine)	1.0	SC	Tranquilization occurs in 5-10 minutes and lasts 1-2 hours; useful when given prior to methoxyflurane anesthesia
Diazepam (Valium) Pre-Anesthetic	5-10 2-10	IM, IP IM	Provides good tranquilization and muscle relaxation but no analgesia; onset of effect in 3-5 minutes; full recovery may require up to 12 hours
Ketamine (Ketaset, Vetalar)	40-50 15-20	SC IV	Duration approximately 15-30 minutes; suitable only for minor procedures; poor analgesia and muscle relaxation when used alone
Telazol (Tiletamine HCl, Zolazepam HCl)			<b>NOT RECOMMENDED</b>

### 2. General Anesthetics

Agent	Dose (milligrams per kilogram)	Route	Comments
Alpha Choralose-1% solution	120 80-100	IV IV	Use only for "physiological" non-recovery preparations; <b>Scientific justification needed</b>
Ketamine	20-60 15-20	IM IV	Heart rate, respiratory rate, and blood pressure are depressed; FSH is suppressed (see under tranquilizers)
Mixture of Ketamine and Diazepam	Ketamine 60 Diazepam 5-10	IM	Heart rate, respiratory rate, and blood pressure are depressed; FSH is suppressed (see under tranquilizers)
Ketamine and Xylanzine (Rompum)	25-50 5-10	SC	Duration of surgical anesthesia (20-30 minutes) is dose-dependent; analgesia adequate for intra-abdominal procedures
Pentobarbital	20-50	IV	Narrow margin of safety; administer dilute (1-3%) slowly to effect; more concentrated solutions or intra-arterial injections can cause vessel damage and lead to occlusion and necrosis; duration of anesthesia is 30-60 minutes but complete recovery may require 1-10 hours; peak effect of the IP dosage reached in 5-7 minutes; light

Agent	Dose (milligrams per kilogram)	Route	Comments
			surgical anesthesia lasts about 120 minutes and complete recovery may take 12-20 hours
Isoflurane	Induction 2-3% Maintenance 0.25-2%	Inhalation	Use a non-breathing system; nonflammable and nonexplosive; can be used for euthanasia

### 3. Analgesics

Agent	Dose (milligrams per kilogram)	Route	Comments
Ketoprofen	1.0	IM	Every 12-24 hours
Buprenorphine	0.1-0.05	IM, SC, or IV	Every 6-12 hours
Butorphanol	0.1-0.5	SC, IV	Every 2-4 hours
Morphine	2-5	SC, IM	Every 2-4 hours
Meloxicam	0.2	PO, SC	Every 12-24 hours

### 4. Miscellaneous

Agent	Dose (milligrams per kilogram)	Route	Comments
Bilateral thoracotomy	N/A	N/A	Secondary method

### 5. Euthanasia\*-Inhalant

Agent	Dose (milligrams per kilogram)	Route	Comments
Carbon dioxide 70-100%	N/A	Inhalation	Only after sedation
Isoflurane	Induction 2-3% Maintenance 0.25-2%	Inhalation	Use a non-breathing system; nonflammable and nonexplosive; euthanasia easily performed in a closed container

\*All deaths are to be verified; a secondary method must be performed to ensure death (e.g., pharmacologic agent, exsanguination, decapitation, or thoracotomy).

### 6. Euthanasia-Physical

Agent	Dose (milligrams per kilogram)	Route	Comments
Cervical dislocation	N/A	N/A	Need IACUC approval; secondary method
Decapitation	N/A	N/A	Need IACUC approval; secondary method
Exsanguination	N/A	N/A	Need IACUC approval; secondary method

## 7. Euthanasia-Chemical

Agent	Dose (milligrams per kilogram)	Route	Comments
Pentobarbital	120	IV	
Potassium chloride	1-2 mmol/kg	IV, intracardiac (IC)	Only after surgical plane of anesthesia

## **VIII. Procedures for Use of Euthanasia Gases**

## **USING EUTHANASIA GASES IN THE VAPHS ARF**

### **General procedure for using the core Operating Room GA147:**

1. Make sure you have all necessary equipment and supplies.
2. Place a fresh absorbent pad on the work area to contain your surgical waste.
3. Confirm the exhaust switch (EF-4 Control) is ON (on the wall beside the sink). The red light will be ON when the switch is turned ON.
4. Close the door to maintain the room with appropriate positive pressure and air flow.

### **Components of the Euthanasia System are as follows:**

1. The Carbon Dioxide gas cylinder is along the wall by the door.
2. The top valve of the cylinder must be turned counterclockwise to open the valve (on) and clockwise to close the valve (off).
3. DO NOT adjust the valve on the regulator.
4. The wall pipe from the gas cylinder delivers carbon dioxide into the euthanasia chamber.

### **To Use:**

1. Line the chamber floor with an absorbent pad. Take care not to block the scavenger outlet.
2. Turn on the scavenging system.
3. Animals may be euthanized by placing their open home cage into the chamber or placing the animals directly into the clean chamber. Secure both latches.
4. Open the top valve on the carbon dioxide cylinder by turning the valve counterclockwise.
5. Open the valve to the carbon dioxide line on the wall behind the chamber to deliver the gas to the chamber. Set the flow meter to deliver the carbon dioxide at a flow rate of 20% (setting 7.5) of the chamber volume per minute. No more than 10 mice, 4 rats and 1 rabbit should be euthanized in the carbon dioxide chamber at one time. Animals being euthanized should be of the same species and similar age.
6. After verifying that the animals are deceased, close the top valve on the carbon dioxide cylinder by turning the top valve clockwise.
7. Remove deceased animals. Note: Very young mice and rats are resistant to the hypoxia-inducing effects of carbon dioxide and thus require longer carbon dioxide

exposure times (e.g., 1 hour) as well as a secondary physical method of euthanasia (e.g., decapitation or cervical dislocation).

8. Open the valve of the carbon dioxide line leading to the chamber until the metal ball falls to the bottom of the meter. This allows the carbon dioxide line to bleed out completely. Turn the flow meter clockwise to close the flow meter. Close the valve to the carbon dioxide line on the wall behind the chamber.
9. Leave scavenging system on to evacuate the gas from the chamber.
10. Put deceased animals in a plastic bag and place bag in -20 freezer.

**Clean-Up:**

1. Remove the absorbent pad(s) and clean up extensively!! Wipe all work surfaces, euthanasia chamber, etc., with an approved disinfectant provided by the ARF.
2. Turn the scavenging exhaust switch OFF.
3. Lock the door when you leave.

## **IX. Procedures for Use of Anesthetic Gases**

## **USING ANESTHETIC GASES IN THE VAPHS ARF**

### **General procedure for using the core Operating Room in GA147**

1. Make sure you have all the necessary surgical equipment and supplies for that day's procedures (such as balance, surgical instruments, lamp, gauze, needles, saline, etc.).
2. Place a fresh absorbent pad on the work area to contain your surgical waste.
3. Confirm the exhaust switch (EF-4 Control) is ON (on the wall behind the set-up area). Red light will be ON when the switch is turned ON.
4. Close the door to maintain the room with appropriate positive pressure and air flow.
5. Ensure the blue hose (Scavenging System) used to remove the Waste Anesthetic Gas (WAG) is connected to the induction chamber and the surgery table.

### **Components of the Anesthesia system:**

1. The compressed gas cylinders are along the left wall.
2. The TOP valve must be turned counterclockwise to open the valve on and clockwise to close the valve off.
3. DO NOT adjust the valve on the regulator.
4. The clear hose delivers oxygen. When using oxygen, set the flow meter to deliver the desired percentage of the gas.
5. The clear hose is connected to the air mixer (far right side as you face it), where the gas is delivered directly to the vaporizer.
6. The VAPORIZER is the machine that adds the anesthetic (isoflurane) to the carrier gas(es).
7. At the top of the vaporizer is the hose that supplies the O<sub>2</sub> to the vaporizer. Check and make sure the hose is connected to the supply tank and is plugged into the vaporizer.
8. The VAPORIZER contains isoflurane; check the level in the machine and add more if needed. A glass funnel assists in pouring the isoflurane into the chamber (Reminder – this must be done under the chemical fume hood).
9. The vaporizer settings for isoflurane are 0.25 to 4.0% + to effect for rats and mice.
10. The vaporizer has an exit hose with a plastic connector to which you can attach the anesthesia induction chamber, anesthesia nose cone, or other apparatuses that deliver the anesthetic mixture to the animal.

11. As noted above, the blue hose must be connected for scavenging the WAG.
12. \*\*For users of nitrous oxide: If the protocol calls for the use of nitrous oxide, all of the steps 2-11 above must still be followed in order.

**To Use:**

1. Open the top valve on the compressed gas cylinder.
2. Turn on the workstation supply line (handset above the vaporizer) for the compressed gas flow.
3. Adjust the gas mixer proportions: set the green knob for oxygen.
4. Put the animal into the anesthesia induction chamber and monitor its breathing.
5. Turn the vaporizer ON. Assure the outlet hose is in the anesthesia induction chamber and allow it to equilibrate.
6. Remove the anesthetized animal from the induction chamber, and attach the outlet hose clip to the operating apparatus.
7. Place the animal on the operating table with nose positioned in the nose cone or other anesthetic delivery apparatus or attach to ventilator, intubate animal and then attach to endotracheal tube.
8. Conduct your surgery.
9. \*\*For users of nitrous oxide: Set the blue knob for nitrous oxide and follow steps 4-8 above in the listed order.

**WHEN PROCEDURES ARE COMPLETE:**

1. Return the vaporizer setting to zero.
2. Close the top valve on the compressed gas cylinders along the wall by turning the valve clockwise
3. Turn off the work station supply lines.
4. The vaporizer gases should be bled off.
5. Turn the flow meter knobs clockwise. The indicator balls will fall to the bottom indicating zero pressure.
6. Turn the exhaust switch OFF.

**CLEAN-UP**

1. Remove absorbent pad(s) and clean up extensively!
2. Wipe all work surfaces, euthanasia induction chamber, etc., with an approved disinfectant provided by the ARF.
3. Turn the scavenging exhaust switch OFF.
4. Lock the door when you leave.

IF YOU HAVE ANY QUESTIONS, CONTACT the ARF SUPERVISOR at 412-360-6107.

## **USING LIQUID (ISOFLURANE) for ANESTHESIA in the VAPHS ARF**

The following rooms are equipped with a chemical fume hood or tabletop ventilated workstation that is appropriate for use with inhalant anesthetic gases: GA146 and GA147. Instructions for isoflurane use in each area are as follows:

### **Room GA146 and GA147:**

1. Make sure you have all the necessary surgical equipment and supplies for that day's procedures (such as balance, surgical instruments, lamp, gauze, needles, saline, etc.).
2. Place a fresh absorbent pad on the work area to contain your surgical wastes.
3. Confirm the exhaust switch is ON (EF-4 Control). Red light will be ON when the switch is turned ON.
4. Close the door to maintain the required air pressure within the room. GA146 should have a negative airflow compared to the hallway and GA147 should have a positive airflow compared to the hallway.
5. Place your anesthesia jar containing absorbent material (cotton, gauze) in the hood. Keep lid/cover on as much as possible to minimize your potential exposure.
6. A 2-4% concentration of isoflurane gas can be achieved in the jar by applying isoflurane at 0.2 ml/1000ml of jar volume to the absorbent material below a false floor in the jar.
7. Anesthesia of mice can be anticipated within minutes of exposure.
8. At the conclusion of your procedure(s), the jar and absorbent material are left under the hood to permit the vapors to disperse to the outside air.
9. Return your stock bottle of isoflurane to its storage area.
10. Remove absorbent pad(s) and clean up extensively!
11. Lock the door when you leave.

## **X: Procedures for Use of Glass Bead Sterilizer**

**F\*S\*T**  
**FINE SCIENCE TOOLS**

INSTRUCTIONS FOR  
Using a Glass-bead Sterilizer  
FST No. 18000-45, FST No. 18000-50

1. Fill sterilizer well with glass beads provided, to within 3 millimeters (mm) below rim.
2. Insert electrical plug into wall socket. Do NOT cover well with lid. Switch on unit. It will take 15 to 20 minutes to reach operating temperature of approximately 250° C.
3. Carefully insert the working part of **clean** surgical instruments into well after unit has reached operating temperature and leave for 10 to 20 seconds. Do not leave longer as instruments become too hot to handle. Sterilization is on contact with glass beads, so tubes (canulae) cannot effectively be sterilized.
4. Unit can be left in the “ON” position all day and is safe for use in laminar flow cabinets.
5. Do not cover with lid when unit is hot. Should unit overheat and trip safety switch, let it cool off completely, then turn it over and push in red button visible inside the unit as viewed through one of the cooling holes.
6. Glass beads can be cleaned by putting dirty beads in a mesh bag with warm soap and water. Rinse and dry beads thoroughly and return beads into sterilizer.

## **XI: Delegation of Authority for ARF Staff**

## **RESPONSIBILITY:**

- Principal Investigators (PIs) are responsible for initiating all requests for animals.
- The ARF Supervisor is responsible for ensuring that there are appropriate resources (i.e., space, equipment, staffing) prior to approving orders for any research animals requested by PIs.

## **PROCEDURES:**

The ordering of all research animals must be coordinated through the VAPHS ARF Supervisor. At times when the ARF Supervisor has been absent for 3 or more days or it is known in advance that the ARF Supervisor will be absent for 3 or more days, the ARF Staff may coordinate the ordering of small mammals (e.g., mice, rats, and rabbits).

PIs must notify the ARF Supervisor in writing of any requests for research animals. Such requests must be sent electronically to the ARF email address [VHAPTHARF@va.gov](mailto:VHAPTHARF@va.gov). Any requests sent to the ARF email address will be automatically forwarded to the ARF Supervisor, ARF Staff, and the Administrative Officer for R&D (AO/R&D). The ARF Staff and/or the AO/R&D will be responsible for responding to and coordinating the ordering related to any requests if 3 or more days have passed since the date of the request and the ARF Supervisor has been absent from work during that time. Additionally, the ARF Staff and/or the AO/R&D will be responsible for responding to and coordinating the ordering related to any requests received when it is known that the ARF Supervisor will be absent from work for 3 or more consecutive days.



## **APPENDICES**

## **Appendix A: VAPHS IACUC SOP**

The VAPHS Institutional Animal Care and Use Committee Standard Operating Procedures may be accessed by via the VAPHS Research Office Website:  
<http://www.pittsburgh.va.gov/Research/docs/IACUC-SOP.pdf>.

**Appendix B: Contamination Control Procedures**  
Example Signage

**ANIMAL RESEARCH FACILITY  
CONTAMINATION CONTROL POLICIES**

**TO ALL PERSONS ENTERING THE ANIMAL RESEARCH FACILITY:**

You must put on new shoe covers every time you enter the facility.

Before entering any animal room, procedure, or operating room, you must put on shoe covers, a yellow isolation gown, and exam gloves. These items are provided for you at several locations in the hallways of the facility.

Some animal rooms may also have additional garbing requirements. These rooms will have the appropriate signage on their doors.

## **Appendix C: VAPHS Waste Anesthetic Gases and Vapors Exposure Control Policy**

The VAPHS Waste Anesthetic Gases and Vapors Exposure Control Policy may be accessed via the VAPHS Research Office website at <http://www.pittsburgh.va.gov/Research/docs/A-001-Waste-Anesthetic-Gas.pdf>.

## Appendix D: Badge Monitoring

### User Instructions:

All employees working with anesthetic gases will be required to wear a personal monitoring badge that measures a person's exposure to waste anesthetic gas (WAG). The badge is used to monitor a single procedure or time frame not to exceed 8 hours. The badges are located on the door of GA119 in a basket along with these instructions. Any questions, please contact the Industrial Hygienist at 412-360-3705.

Instructions for use of the badges and required documentation:

1. To prevent damage to the resealable seam, cut an opening at the top of the white Ziploc package, above the notched area.
2. Remove the monitoring badge from the white Ziploc package and then remove from the clear resealable plastic bag. Save both the outer white Ziploc package and the clear resealable plastic bag.
3. Record the Date, the Start time of your procedure, and the Name of the Technician who is actually doing the procedure on the badge and on the outer white Ziploc package.
4. Clip the badge near the breathing zone (collar or lapel). Make sure the white, blank surface is facing away from the body so it is exposed to the gas. Make sure the white side of the badge is exposed to the gas throughout the entire procedure.
5. When the procedure has been completed, remove the badge from the breathing zone and record the Stop Time on the badge and on the outer white Ziploc package. Also somewhere on the outer white Ziploc package, record the room number where the procedure was performed, the number of animals used, the type of anesthetic gas used and a general description of the procedure. Examples of procedures would include:
  - Clipping and shaving the mice
  - Any surgical procedures
  - Euthanasia of the animal
6. Place the exposed monitoring badge into the resealable plastic bag and then into the white outer Ziploc package and seal. Make sure the seal is tight.
7. After completion of the procedure and all documentation, place sealed badge in the receptacle located outside of GA119 to be submitted for exposure results.

## **Appendix E: VAPHS Animal Exposure Preventive Medicine Program**

The Animal Exposure Preventive Medicine Program (AEPMP) policy may be accessed via the VAPHS Research Office Website at: <http://www.pittsburgh.va.gov/Research/docs/A-002-AEPMP.pdf>.

## **Appendix F: Employee Injury**

All employee work-related injuries are handled in accordance with VAPHS Medical Center Memorandum HR-023, On-The-Job Injury Response. This document can be accessed from the VAPHS SharePoint site.

## **Appendix G: Guidelines for Multiple Major Survival Surgeries**

### **Purpose**

The purpose of these guidelines is to provide guidance to PIs who perform multiple major survival surgeries.

### **Background**

Both USDA Regulations and the Guide for the Care and Use of Laboratory Animals address this issue in terms similar to those used above in the ‘Purpose’ of this document. “Major surgery” is defined as surgery that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological function. A second major surgery in which the animal is euthanatized without recovering from anesthesia is not considered to be multiple major survival surgery.

### **Guidelines**

Multiple major surgeries on the same animal are discouraged. If it is proposed, the PI must justify its use either for scientific reasons, or the other reasons listed above under “Purpose”. Multiple major surgeries cannot be justified by cost savings alone. It is the obligation of the IACUC to evaluate any justification of multiple major survival surgery, to communicate their determination to the PI, and to further consider any disagreement(s) that exist between the PI and the IACUC.

## Appendix H: Guidelines for Anesthetics and Analgesics Use in Rodents

The following drugs and combinations of drugs are recommended by the VAPHS IACUC. All drugs proposed to be used in animals must be described in the ACORP and approved by the IACUC. Drugs of these categories as well as combinations of them that are not listed in this document can be used when approved by the IACUC.

### Anesthetics:

#### **Inhalants**

Inhalants are preferred because of the ease of controlling depth of anesthesia and the brevity of recovery. However, the animal's vital signs must be monitored to avoid an overdose or light anesthesia. Reliable vaporizers must be used for the effective delivery of volatile anesthetics; oxygen or a mixture of oxygen and nitrous oxide are the carrier gases of choice. The combination of oxygen and nitrous oxide can be used at 30%/70% or 50%/50% respectively. Humans must not be exposed to the vapors of inhalant anesthetics.

Isoflurane: Induction is best done in a chamber using 4% isoflurane. Most rats and mice can usually be maintained in surgical anesthesia using 1 to 3% isoflurane, but it is used to effect.

#### **Injectables**

Injectable anesthetics are more difficult to titrate than inhalants and usually result in a longer recovery period. However, they have the advantage of ease of administration without WAG exposure concerns. **Examples of some standard anesthetic regimens include:**

1. Ketamine: By itself, ketamine should be used for restraint and for minor procedures as it usually does not produce deep anesthesia and causes muscular rigidity. However, in the following combinations, it can be used for major surgical procedures in rodents:
  - A. Ketamine and Xylazine (respectively)  
Rat: 40-80 mg/kg and 5-10 mg/kg IM  
Mouse: 80-120 mg/kg and 5-15 mg/kg IM
  - B. Ketamine, Xylazine, and Acepromazine (respectively)  
Mouse: 30 mg/kg, 6 mg/kg IM, and 1mg/kg IM, IP
2. Sodium Pentobarbital:  
Rat: 40-50 mg/kg IP to effect  
Mouse: 40-90 mg/kg IP to effect. It is recommended that ketoprofen be given at 5 mg/kg SC for supplemental analgesia; other analgesics can be used, but some cause additional respiratory and cardiac depression.

## **Analgesics**

It has been shown that animals, including humans, recover faster from surgery when postsurgical analgesics are used. Analgesics are most effective if they are used preemptively in effect before the pain starts. Analgesics are to be used post-surgically, except for very minor procedures, and when other painful situations are expected unless there is a scientifically justified reason that is approved by the IACUC.

1. Ketoprofen:  
Rat: 5.0mg/kg, SC every 12hrs  
Mouse: 5.0 mg/kg, SC every 12 hrs
2. Buprenorphine:  
Rat: 0.01 to 0.05 mg/kg, SC, IM, IV every 8-12 hrs  
Mouse: 0.05 to 0.1 mg/kg, SC or IM every 12 hrs
3. Butorphanol:  
Rat: 2.0 mg/kg, SC, IM every 4 hrs  
Mouse: 1 to 5 mg/kg, SC, IM every 2-4 hrs
4. Morphine:  
Rat: 2.5 mg/kg, SC every 2-4 hrs  
Mouse: 2.5 mg/kg, SC every 2-4 hrs
5. Meloxicam:  
Rat: 0.2 mg/kg, SC, PO every 12-24 hrs  
Mouse: 0.2 mg/kg, SC, PO every 12-24 hrs

## **Appendix I: Animal Research Facility Emergency Operations Plan**

The Animal Research Facility Emergency Operations Plan can be accessed via the VAPHS Research Office website at: <http://www.pittsburgh.va.gov/Research/docs/ARF-emergency-operations-plan.pdf>.