Standard Operating Procedures (SOP)

I. General Information

A. It is the responsibility of all individuals working in the animal facility and conducting research in the ARF (Animal Research Facilities) to be familiar with the contents of this Standard Operating Procedure (SOP) manual. Any actions that occur that are in violation of these procedures will subject the individual as well as the Principal Investigator's entire animal care program to ARF SOP violation. Thus, it is important to read and be familiar with the document in Appendix pertaining to SOP violations.

B. Operation of the ARF is the responsibility of all Principal Investigators and employees. All individuals using the facility must maintain cleanliness, humane treatment of animals, complete records, and proper respect for the rights of all personnel. Principal investigators are required to provide the animals with proper housing, handling, feed and water while in the facility. Health monitoring will be done daily, including weekends and holidays.

C. This SOP serves as a guideline for the housing and care of laboratory animals in the ARF and serves the following functions:

1. Assurance that all personnel follow the same procedures.

2. Training document for new personnel and reinforcement of procedures for established personnel.

3. Reference source of information about standard procedures.

D.lt is the responsibility of the Facility Supervisor to oversee the daily operations of the animal facility and assure compliance with the SOP outlined in this document.

E. Charges & billing

The Principal Investigator will receive an invoice via email fortnightly or monthly from ARF to cover the per diem, bedding, food and animal cost. Bills should be paid in a timely manner.

F. Access Control System

The ARF has an access control system for ensuring safety of the animals. This system consists of electronic cards which provide access strictly to people who work with animals: researchers, technicians and employees working in our facilities.

The IT department is responsible for providing the cards and fixing the system in case there is a malfunction with the cards.

G. Joint-use space (Preparation Room) is available for all Principal Investigators with the approved animal room space on a first-come, first-served basis. It is expected that Principal Investigators and their staff will return all joint-use space to its original clean and neat condition as soon as they have completed their use. Reservations may be made by calling the Animal House Supervisor. The joint-use room may be

reserved up to one month prior to the time it is needed. The Facility Supervisor will mediate any conflicts over joint-use space.

H. Freezer is available for all users of the facility. All items placed in the freezer must be marked with: (1) contents; (2) date placed in storage; (3) the Principal Investigator's last name; (4) number of animals per bag.

I. Biosecurity Issues:

- a. Personnel must follow the guidelines of not entering any animal room that has a higher biosecurity rating (i.e., B, C, D; being highest to lowest biosecurity rating, respectively) after being in a room with a lower rating. For example, if you have mice in a "B" and "C" room, personnel should complete their work in the "B" room before entering the "C" room. Personnel must undergo a complete change of clothes before returning to a room of higher biosecurity.
- b. The Facility Supervisor must be informed beforehand about plans to bring in equipment from other animal facilities. Such equipment must be disinfected prior to or immediately upon arrival.
- c. In order to reduce the risk of spreading infectious disease, it is the facility policy that animals may not be returned to the facility after leaving the building. Exceptions to this policy may be permitted only if specified in the animal protocol and approved by the Facility Supervisor and the IACUC committee.
- d. Individuals that work with laboratory animals elsewhere on campus or outside should prepare a plan for how they will transport the animals between the ARF and these other facilities. The plan must be approved by the Facility Supervisor in consultation with the IACUC committee.
 - i. The vehicle must have an air conditioning system.
 - ii. It also has to have enough space in the cabin to allow good ventilation inside the transportation cages.
 - iii. The animal must be awake in a transportation cage that complies with the required space, in accordance with AW A.
 - iv. The animal must be accompanied always by a well-trained research technician.
 - v. The animals should never be left alone inside of the car without proper ventilation. This is important given the strong heat, sun and dryness in Ponce.

J. Security: Access to the ARF will be limited to authorize personnel only. Authorized personnel (i.e., those with ARF security cards in their name) must accompany all visitors at all times while they are in the facility. The Principal Investigator does not need to seek permission to bring visitors into the facility. We

only seek to be kept informed about when unauthorized personnel are in this facility, and who is responsible for them during their visit. Instructors who will need access to the facility for instructional activities should provide the Facility Supervisor with the name of the course, and dates students will be using the facility. We do not want this to be an impediment to the use of this facility for teaching purposes, since this is one of the missions of the Facility.

K. Housing

a. The cage size should allow for the normal postural adjustments of the species involved.

b.<u>The Guide for the Care and Use of Laboratory Animals</u> sets limits on the maximum number of animals that can be housed in any primary enclosure. This number varies with the size of the cage and the weight of the individual animals, and is not to be exceeded. See the SOP for specific animals for additional details (Appendix II) or consult the "Guide".

c. Only one species of animals is to be housed in an animal room. Animals should be segregated by sex and caged accordingly, unless a specific experimental procedure, as described in an approved IACUC protocol, dictates otherwise.

L. Water: All animals must have access to clean, potable water at all times (ad libitum) unless approved experimental procedures dictate otherwise and have received IACUC approval.

- 1. Water bottles are replaced at least once per week, or more often if necessary to assure access to clean potable water. Between bottle changes, water bottles may be topped off, but otherwise the bottle should be changed rather than refilled. Sipper tubes and toppers are sanitized in the cage washer.
- 2. When mice are housed in plastic cages, care must be taken to ensure that sipper tubes are long enough and no curved sipper tubes are used.

M. Feed: All animals must have access to food at all times (ad libitum) unless experimental procedures dictate otherwise and have received IACUC approval. The food must be clean and free from contaminates.

- a. Storage of food must be in a portable container with lids.
- b. Feed containers must be sanitized once a month.
- c. The date of setting up a new feed bag in the container must be documented on the feed records.
- d. Feed purchase will be placed with supply service early enough that food is always available.
- e. Unopened feedbags with a milling date of over 9 months must be discarded.

N. Electrical energy: Our facilities have a power plant which is active between 15 to 30 seconds after a power outage. The power plant is serviced once a month to ensure its operation.

0. Transportation: Animal transportation should not be undertaken if an animal is likely to be exposed to temperature extremes. All cage furniture or food and water receptacles that are not fixed must be removed before transportation.

All reasonable attempts should be made to minimize stress to animals which may include:

- a. Transporting with cage mates
- b. Transporting in home cages (if secure)
- c. Provide a nutrition/water source
- d. Low noise
- e. Minimum duration; may be exceptions, subject to compliance with other requirements

Procedure:

- 1. Only specially constructed animal transport cages should be used.
- 2. Road transportation should be undertaken in air conditioned vehicles by accredited animal transport providers or trained animal technicians.
- 3. Cages must be secured in the vehicles.
- 4. Transport time should be minimized and animals re-housed into appropriate cage groups as soon as possible.
- 5. Caution should be exercised when mixing adult males for transport. Animals should be monitored for aggression and re-housed into separate cages on arrival.

P. Air Quality: The air system provides 15 fresh air changes per hour to maintain macro-environmental air quality by constant volume system and may also ensure micro environmental air quality.

II. ARF Responsibilities to other Organizations

A. Institutional Animal Care and Use Committee (IACUC)

Each institution falling under the PHS or the Animal Welfare Act regulations is required to have an Institutional Animal Care and Use Committee of a prescribed composition, and to assign the IACUC specific responsibilities. The Ponce Health Sciences University Animal Care and Use Committee meet the prescribed membership requirements and are charged by the Institutional Official with a specified set of responsibilities, which include:

- 1. Review of animal care and use programs every six months.
- 2. Inspection of animal care and use facilities every six months;
- 3. Preparation of reports of the animal care and use program and facility evaluations;
- 4. Making recommendations to the Institutional Official regarding any aspect of the PHSU animal program, facilities or personnel training;

- 5. Reviewing and approving, requiring, modifications in (to secure approval), or withholding approval of proposed and ongoing protocols involving the use of animals in research;
- 6. Recommending suspension of activities involving animals that are not in compliance with existing standards, guidelines, and regulations;

7. Reviewing concerns involving the care and use of animals at PHSU. Appendix III: Ponce School of Medicine D16-00352 Animal Welfare Assurance for Domestic Institutions

B. The Public Health Service

The PHS requires that institutions, including PSMHS, receiving PHS funding assure the humane care and use of animals regardless of funding source. This compliance takes the form of an Animal Welfare Assurance statement filed with, and approved by, the Office of Laboratory Animal Welfare. The PHSU assurance includes a statement of policy complying with appropriate laws and guidelines; a listing of facilities; descriptions of programs for animal care and use, occupational health, and training of personnel who work with animals; and a description of the IACUC, its functions and composition.

III. Personnel Training & Responsibilities

- A. It is responsibility of the Principal Investigator and Facility Supervisor to provide adequate personnel to properly maintain his/her animals during both the work week and weekends, including holidays. Proper maintenance of animals includes: feeding, watering, cage changing, cage washing, and room sanitation.
- B. The ARF has a training program that is mandatory for all individuals that are new to working in this animal facility. Workshops will be offered every four years for all personnel using animals.
 - 1. Individuals with animals are required to complete the training prior to receiving security access to facility and before beginning work in the facility. The Facility Supervisor is always available to provide hands-on training as needed for all animal users. All training are documented.
 - 2. Gloves and clean lab coats must be worn in the animal rooms.
 - 3. Animal care personnel are to work in their assigned area and avoid indiscriminate visits to animal rooms outside their responsibility.
 - 4. Eating and drinking are not permitted in animal rooms, preparation room or hallways. Food and drinks intended for human consumption must be stored and consumed in the break room or Supervisor's office.
 - 5. All persons in contact with animals must wash their hands thoroughly upon entering and leaving animal rooms. This is for the protection of the employees as well as the animals.

- 6. The ARF has CD's and handbooks for the training of personnel working with animals. The following resources will be available in the Facility Supervisor's office and/ or in the IACUC Chair's office:
 - a. Handbook of Clinical Signs in Rodents and Rabbits Published by Charles River Laboratories, 2nd Edition, April 2011.
 - b. CD of Training in Survival Rodent Surgery" Produced by NIH Office of Animal Care and Use and National Humane Genome Research. 2011.
 - c. CD of "Mouse & Rat Humane Restraint and Experimental Technique, 2011.Produced by NIH Office of Animal Care and Use and National Humane Genome Research 2011.
 - d. CD's of Animal Training Workshops held at PSMHS: Wet Lab on Animals Well Being, 2002 and Wet Lab on Rats Handling 2004.
 - e. There are additional texts available that can be helpful related to animal use. See Appendix IV for more details.
- C. **Bites & injuries:** Any employee bitten by an animal or injured on the job must report the incident immediately to their supervisor. The individual injured is encouraged to seek medical attention even for minor injuries. All injuries require completion of accident reports within 24 hrs. of the injury. Instructions and forms are available in the Human Resources Office in the PHSU Administration Building.

IV. Procurement

A. Animal Procurement

The Facility Manager and/or Principal Investigator places the orders for animals to be housed in the Facility. Requisitions will be made under Jenzabar Program of the Institution. Requisitions will be made as follows: Facility Supervisor and /or Principal Investigator -Department Director- Budget Department- Finance Department- Purchasing Department.

Every researcher must follow the following steps to request animals:

Enter the site: <u>www.psm.edu</u> - Research - Animal Research Facilities-Animal Requisition Form - Submit.

This application comes to the Office of the Facility Supervisor where she/he verifies the availability of the animals and proceeds to answer the application.

Principal Investigators may not order animals unless:

I. They have an approved and up-to-date Animal Care and Use Protocol on file with the Facility Supervisor;

2. They have an approved request for space in the facility;

3. Animals are from an approved source or have undergone quarantine;

4. Animals will be procured only when adequate cages and space are available.

B. Supply Procurement

I. Food and bedding will be ordered and purchased by the Facility Supervisor and stored in the Storage Room.

2. Supplies such as cleaning supplies, paper towels, trash bags, etc will be ordered and purchased by the Facility Supervisor.

3. The Principle Investigator will be responsible for ordering special diets.

Our facility has available also the sale of food and bedding.

The requisitions will be made in the following order:

Enter the site: <u>www.psm.edu</u> - Research - Animal Research Facilities - Animal Requisition Form - Special Requirements - Submit

C. Equipment Procurement

1. The initial inventory of caging and equipment in the ARF was accumulated through purchases with start-up money. The facility, Principal Investigators, and the Research Incentive Fund have paid for subsequent purchases of new equipment. This included equipment needed for specific projects and replacement of equipment due to wear and tear from long-term use.

2. The ARF's policy is that any caging used in the ARF regardless of its source, becomes property of the ARF and is made available for shared use, unless special arrangements have been made in advance. An example of an exception might be equipment borrowed from another facility on campus.

The following (see list below) are sources of laboratory animals routinely used by the ARF. Other sources are used at the Principal Investigator's discretion.

List of Approved Animal Sources/Venders

Rats/ Mice

Charles River Breeding Laboratories, Inc. 251 Ballardale St. Wilmington, MA 01887 (508) 658-6000 (800) 228-4919 FAX: (800) 255-8964

ENVIGO. P.O. Box 29176 Indianapolis, IN 46229 (317) 894-7521 FAX: (317) 894-1840

Taconic Farms, Inc. 273 Hover Avenue Germantown, NY 125 26 (518) 537-6208 or (888) TACONIC FAX: (518) 537-7287

Jackson Laboratories, Inc. Bar Harbor ME 04609 (207) 288-3371; (207) 288-5845 (800) 422-MICE (for orders only) Fax: (207) 288-3398 Hilltop Lab Animals Inc. Hilltop Drive, Scolldalle Pensylvania 15683 (724) 887-8480 (800) 245-6911 Fax (774) 837-3582

V. Animal Health Monitoring

A. Daily Health Checks: The condition and welfare of all animals MUST be monitored daily and noted on the Animal Health Observation sheet posted inside the animal room. Checking the conditions of bedding and food, availability of water, and health of the animals are to be considered essential duties of animal care personnel, technicians and PIs.

1. Animals MUST be observed daily, with the Animal Health Observation sheet filled out and signed.

a. Any animal showing any abnormality (e.g., grossly sick, injured, lame, circling, head tilt, loss of appetite, etc.) MUST be noted on the Animal Health Observation sheet; Report abnormalities even though they may seem to be of minimal significance, and regardless of whether experimentally-induced or spontaneous;
b. Next, the Principal Investigator or the Facility Supervisor MUST be notified;

c. While anyone can initiate a call to the Attending Vet, the Principal Investigator is ultimately responsible for making this determination for all animals assigned to his/her research program and IACUC protocol(s);
d. Contacting the Attending Vet Mónica Montalvo: During normal business hours (8 am-4:30 pm); use number (787) 450-1489.

2. Dead Animals: Any animal found dead MUST be recorded on the Animal Health Observation Sheet.

a. Upon discovery of an unexpected dead animal, a determination should be made regarding whether the animal might be suitable for necropsy. Such a determination should be made by the Principal Investigator, Facility Supervisor, or Attending Vet.

b. Dead animals are to be placed in a clear plastic bag (available in garbage/freezer room).

c. If the dead animal is part of a research experiment, the investigator in charge will be notified and ask whether to proceed for necropsy. In -House necropsies are considered "gross-post-mortum". If the researcher elects cultures or histopathology, the samples will be sent to an offsite laboratory and charges might apply.Bagged animals are required to have a tag affixed to the bag with appropriate information filled out: name of PI, room#, date and animal #.

d. If the dead animal will not be submitted for necropsy, then it should be taken to the freezer in the garbage/freezer room. The Facility Supervisor will see to it that the dead animal is disposed of properly. **Appendix V**: **Disposal of Dead Animals**

B. Escaped Animals: Animals found outside a cage are, if possible, to be captured and then placed in a clean cage separate from other animals. Under most circumstances, such animals should be humanely euthanized and not returned to the colony. Exceptions to this policy can be made under some circumstances, but only after consultation with the Facility Supervisor. **If** the animal is under experiment, the technician and / or principal investigator will be notified and they will decide what to do with the animal.

VII. General Animal Care Procedures

A. General: The purpose of this SOP is to describe general procedures applicable to the routine husbandry of animals housed at the ARP. The animal care staff should become familiar with this SOP.

B. Animal Care: All animals housed in the facility are to be housed in, secure quarters and have feed and clean, fresh water. Sanitation of animal caging and rooms is of primary importance in preserving animal health and comfort. The sanitation measures prescribed will be rigorously followed. The animal care staff observations of animal health and housing conditions are of critical importance and receive a high management priority. Animals will be handled only by prescribed methods, which minimize stress and discomfort, and maximize safety to both the animals and the handler.

C. Cage card information: All cages housing animals MUST display a cage card containing the following information: (1) species; (2) number of animals in cage; (3) Principal Investigator's name; (4) source from which animals were obtained; (5) date of arrival or birth. The facility uses a color code for identification of animals as

follows: blue-males, pink-females-, yellow-litter, orange-pregnant, green- breeding and white- experimental animals and/or sick animals.

D. Room card information: All animal rooms will display a room card on the door. A room card should be present for each research project. This card is to identify the Principal Investigator, protocol number, species of animal, responsible individuals and their phone numbers both at work and at home.

E. Shipments: All shipments for the Animal Research Facility are to be checked in with the ARF Supervisor upon arrival. Refer to the section on "Procurement" for more details regarding how animals and supplies are to be ordered.

VIII. Procedures for Incoming Animals

A. Facility Supervisor and/or Principal Investigator are responsible for placing all animal orders associated with research projects housing animals in this facility.

1. Commercial suppliers with known clean health status may supply animals to Investigators- animals will be in quarantine for 10 days.

2. Animals from other, non-approved, sources (e.g., Investigators at other universities) must be approved by the Facility Supervisor. Principal Investigators are required to provide the Facility Supervisor with a copy of the satisfactory health status report BEFORE these animals are brought into this facility- animals will be in quarantine for 15 days.

B. Health monitoring reports for all rodents from non-approved sources are reviewed by the Attending Veterinarian. Depending on the information provided in these reports, these animals may be quarantined and diagnostic samples submitted as needed until satisfactory animal health reports are obtained. All costs associated with quarantine and screening will be the responsibility of the Principal Investigator.

C. Rodent shipping boxes MUST be checked by the animal care personnel for damage and dead or sick animals as soon as possible, but no longer than 2 hours after delivery to the facility. Dead and sick animals MUST be removed from shipping boxes and the Facility Supervisor notified so that the supplier and shipping firm may be notified.

1. Ordinarily, rodents should be housed as soon as possible upon arrival (i.e., within a few hours).

2. If this is not possible, then they should be placed in quarantine room while still in their shipping containers.

3. Water should be provided to the animals in the shipping box. This can be accomplished by placing a clean water bottle on the top and pushing the sipper tube through the screen.

D. Acclimation/Conditioning: It is recommended animals should be allowed a minimum period of one week for conditioning and health status evaluation before assignment to experiments. Incoming animals suspected of being sick should be

isolated from other animals. The Facility Supervisor is to be notified of this condition and the Attending Veterinarian contacted.

IX. Cage Changing Procedure for Contact Bedding

A. Bedding in direct contact with animals is to be changed a MINIMUM of once a week. Heavily soiled bedding should be changed more often.

B. The following procedure is recommended for changing cages with contact bedding:

1. Count number of cages that need to be changed.

2. Take desired number of cages from the clean storage area and place them on a clean cart.

3. Fill cages with bedding of a sufficient quantity to insure comfort of the animals and provide adequate absorbance of animal waste. As a guide, it is recommended to cover the entire cage bottom to a minimum depth of 1 cm for cob-type bedding and 2 cm for aspen shavings.

4. Transfer cage cards, food hoppers, water bottles, and animals into clean cage, one

- at a time and replace cage at the same location on the rack.
- 5. Wire lids should be changed every two weeks.
- 6. Micro-isolator tops, if used, should be changed once a week.
- 7. Racks should be wiped down with a sponge while changing cages.
- 8. Record what was changed on room folder.

C. Remove dirty cages and equipment to dirty cage wash area. Do NOT leave dirty cages in hallways for more than a short time (e.g., a few hours) or in the animal rooms for more than a day (i.e., 24 hrs).

D. Dump soiled bedding in a bag-lined dumpster/garbage can.

Any food, bedding, or excreta remaining in the cage MUST be thoroughly scraped free. When finished or when garbage liner is two-thirds to three quarters full, then close liner tightly and dispose of it in the dumpster located outside the loading dock.

E. Pre-wash ALL cages and equipment. Personnel MUST wear eye protections during this process. Be sure to remove all tape and cage cards.

F. Stack equipment (e.g., cages, lids) GENTLY and neatly in dirty cage wash area along the wall nearest the cage washer.

G. Sweep floor around area.

X. Room Maintenance

A. Floor cleaning schedule

1. All floors in animal rooms are to be swept daily with brooms provided.

2. The entire floor in the animal room is to be mopped with a disinfectant solution twice a week. Make sure the proper dilution is used (e.g., TOO MUCH bleach is bad for the floor finish).

3. A clean mop bucket must be used for this. These are available in the clean cage room.

4. Mop buckets are to be returned in a clean condition (i.e., emptied and rinsed out!) in the cage wash area.

B. Sink cleaning schedule

1. Sink and counter area are to be cleaned weekly with disinfectant solution and kept free of clutter.

2. Scale remover should be used as needed to remove scale around faucet.

3. Hand soap and paper towels should be placed in dispensers as needed.

C. Walls, Ceilings, etc. cleaning schedule

1. Door handles, will be sanitized once a week with a disinfectant solution.

2. A hand sprayer is available for spraying these areas. (70% alcohol solution) 3.When a project is completed, animals are to be removed from the room; the room is to be thoroughly sanitized with disinfectant leaving a clean room for the next project.

4. Rooms that have continuously running projects should thoroughly clean walls, ceiling, fixtures, etc. at least once a year. The Facilities Supervisor will periodically designate a time for this during the year.

D. Any concerns about the environment (e.g., temperature, humidity, leaking faucet, etc.,) in any rooms within the Facility should be reported to the Facility Supervisor in a timely manner.

1. Timers are set for each animal room to provide automatic light control. Users are to contact the Facility Supervisor for making any change in the setting of light timers.

2. Burned out light bulbs should be reported to the Facility Supervisor who will arrange to have them replaced by PSMHS maintenance staff.

E. Air filters: should be cleaned by the animal care staff once a month or more often if deemed necessary by the Facility Supervisor. The filters should be changed once every six months.

XI. Cage Pre-Wash Procedure

A. Pre-washing equipment

1. DO NOT bang cages. Use scrapers to remove bedding from dirty cages.

2.All equipment needs to be thoroughly pre-washed before being run through the cage washer. The cage washer is built to sanitize but not remove gross soil and dirt. All tape must be removed from equipment during pre-washing procedure.

3. Do not wash large particles of bedding into the drain of the pre-wash area. This will clog drain and back up onto the floor.

B. Protective clothing

1. Eye protection, gloves, lab coat, and appropriate shoes (i.e., no sandals, flipflops, or open-toed shoes) MUST be worn during cleaning activities.

C. Sanitization of Equipment

(NOTE: The cage washer is to be operated by ARF personnel only unless prior arrangements have been made and training has been provided.)

The operations in wash room should cover the cleaning of most non-surgical equipment including cages, pans, boxes, water bottles, etc. The room is to be operated with one end for dirty items, while the other end contains clean items.

1. Loading cages

a. Shoebox style cages need to be loaded on cage washing rack so they are positioned for maximum exposure to spray from the water jets in the cage washer.

2. Cage accessories, bottles, sipper tubes etc.

a. All cage accessories must be washed in a manner that prevents small items from falling on floor of cage washer.

b. The baskets used for washing water bottles can hold small items.

c. Carts and dumpsters should be washed daily.

3. Manual Sanitization

a. Caging systems, which cannot be washed in the cage washer, must be manually sanitized.

b. Scale from urine and water must be removed with scale remover.

c. Equipment should be cleaned with disinfectant and hot water.

4. Use of cage washer

a. The automatic cage washer must have the wash tank filled before operation. This is done by opening the hot water valve inside of the machine.

b. Before attempting to fill the tank, be sure the wand which drain the tank marked "open-shut" in front of the machine, is closed.

c. In order to use the machine, the main hot water valve and the main disconnect switch located nearby must be turned on. This allows the electric solenoid valve to operate, which allows steam to heat the tank.

d. This machine is adapted with a synchronized detergent and rinse feeder system. To operate the machine the final rinse water valve turned on automatically. In addition, adequate soap should be added by automatic feeder.

e. Operation of the machine should start after the water temperature in the wash tank reaches 180° F.

f. Shut doors and start wash.

g. Equipment should be taken out on the clean side of cage wash area.

h.When operation of the cage washer is completed, the machine should be turned off.

i. The water valve and main disconnect switch must turned off.

j.The water tank must be drained immediately and washed with a hose.

5. Maintenance of cage washer

- a. Clean loose parts from under washer floor to avoid clogging pump.
- b. Twice a week the machine should be sprayed with stainless steel cleaner.
- c. Problems should be reported immediately to the Facility Supervisor.

XII. Euthanasia

A. Animal termination will be carried out in a humane manner by animal facility staff and / or the Principal Investigator or others designated by the Principal Investigator.

1. CO2 chambers located in Washing Area and Preparation Room are available for euthanasia of animals.

The use of this inhalant is recommended in small animals such birds, guinea pigs, rabbits, mice, rats and some low weight cats. This method is used in a closed container. A chamber with a bag inside will be located in a room with a separate ventilation system and connected by a hose to the CO2 gas cylinder. Once the animal is in the chamber it is closed completely. The valve is opened slowly during one minute. Afterwards, the chamber is open and the animal disposed of as

per established procedure for handling dead animals. Appendix V: Disposal of Dead Animals

3.Cervical dislocation, this method is performed only by trained personnel and is used to euthanize mice and rats. The thumb and index finger are placed on either side of the neck and the base of the skull and pressed. With the other hand the base of the tail is quickly pulled. The thumb and the index finger can be substituted by a stick or pencil. Then procedure for handling of dead animals should be followed.

4.Other methods of euthanasia are allowed once they are approved by the IACUC according to the type of investigation.

Summary of Animal Care and Cleaning Schedules

A. Daily

- 1. Check condition and welfare of animals. Fill out Animal Health observation sheet.
- 2. Check all cages for adequate feed and water.
- 3. Sweep floor of all debris.
- 4. Fill sheet for cleaning completed that day, either by the project technicians or the Animal House staff.
- 5. Empty dehumidifiers.

B. Twice a week

- 1. Change direct contact bedding cages.
- 2. Mop floors.
- 3. Wash cages, bottles etc. in the washing machine.
- 4. Empty trashcan.

C. Once a week

- 1. Change micro isolator filters, if used.
- 2. Clean all door handles, sink and counters with alcohol (70%)

D. Once a month

- 1. Clean air filter.
- 2. Sanitize food storage containers.

E. Once every six months

1. Change air filter.

Appendix I: Guidelines for Handling S.O.P. violations in the ARF

Introduction:

Animal facilities in the PSMHS are operated under IACUC supervision. We strive to provide quality facilities for housing research and teaching animals. In order to maintain a quality animal care program it is essential that users follow our Standard Operational Procedures (SOP). Individuals who fail to follow the SOPs place at risk the health and well-being of workers and animals, as well as the biosecurity of the facility. Consequences for violating the SOPs may include charges for Unit support staff to provide unmet animal husbandry needs and loss of privilege to use the facility. The following guidelines will be used for handling violations of SOPs.

In an effort to acknowledge that not all failures to follow SOPs are the same, we have established three levels of violations: "A, B and C" separately within the ARF.

ARF

I. Levels of Violations:

A. Level "A" violations are generally considered minor violations in procedure that do not significantly impact the health and well-being of animals or personnel. Examples of such violations include:

1. Failure to sign the health check sheet in the room;

2. Failure to change cages according to the schedule required by the SOP;

3.Not filling out an Animal Health "WHITE card" despite doing what is needed to assure that appropriate care has been provided to a sick animal;

4. Not recording cage changing on Daily Activity Sheet.

B. Level "B" violations are generally considered to have the potential to put the health and well-being of animals or personnel at some increased risk or may compromise the biosecurity of the facility.

Examples of such violations include:

- 1. Failure to conduct a daily health check;
- 2. Use of diets beyond the six months after the milling date on the bag;

3. Not changing cages or trays as scheduled;

4. Trafficking into the "clean" room after being in the dirty room;

5. Leaving dirty cages in the hallways after leaving the facility;

6.Failure to wear a lab coat or some protective outer clothing during any work that involves direct contact with animals or waste-contaminated products;

7. Failure to clean a joint-use space following surgery, sample collection or animal kill;

8. Propping doors open during any activity that is likely to generate aerosols of waste contaminated products (e.g., bedding, hair);

9. Bringing animals into the facility without informing the Facility manager beforehand.

C. Level "C" violations are the most serious and are considered those departures from SOPs or IACUC protocols that may clearly result in adverse effects on the health and well-being of animals or personnel in the facility or clearly compromise the biosecurity of the facility. Examples of such violations include:

1. Failure to adequately monitor animals following surgery or other invasive procedure;

2. Failure to provide appropriate veterinary care for a sick animal;

3.Failure to provide animals with adequate food and water, unless such restriction is part of the approved IACUC protocol;

4. Failure to euthanize an animal that is clearly in pain and distress, unless specific exceptions have been described in the IACUC protocol;

5. Bringing an animal into the Facility from a non-approved source without going through a standard quarantine;

6. Bringing animals into the facility without an approved IACUC protocol;

7.Bringing animal into the facility from another location (i.e., animal room) on campus without prior approval of the Facility Supervisor.

II. Appeals:

A. If a PI wishes to appeal a specific violation, then they should do so in writing to the Facility Supervisor or IACUC Chair within 7 days of the violation;

B. Depending on the nature of the violation, an appeal may require nothing more than interviewing the parties involved prior to initiating an IACUC investigation into the incident.

III. Administration of Violations:

A. Violations can only be given out by the Facility Supervisor.

B.Personnel responsible for level A violations will be given a verbal warning initially, followed by a written warning for a second violation with e-mail to the PI and Facility Supervisor.

C. All level B violations will result in a written warning with Pls and the Facility supervisor receiving copies of all written warnings;

D. All level C violations must be reported to the IACUC Chair, Supervisor and the PI as soon as possible.

IV. Probation:

A. Anyone who receives three or more written warnings for "Level A" violations or two or more "Level B" violations will be placed on probation.

B. "Level C" violations will be handled on a case-by-case basis by a committee consisting of the Facility Supervisor, and the ARF Attending Veterinarian.

C. Probation will last for **NO LESS THAN A MONTH**; probation means that the individual on probation will not have any privileges associated with working with animals in the Facility, unless directly supervised by someone else qualified & currently authorized to work with the animals.

1. After the second week of probation the employee may conduct all of their work without supervision, if it is conducted during standard working hours (e.g., 8-4:30 pm, M-F only).

2. The probationary period will end at the discretion of the Facility Supervisor after receiving a written request by the PL. A successful petition will clearly describe the additional training that the individual received to assure that every effort has been made to reduce the likelihood that further violations will occur.

3. Additional level A or B violations by someone who is on probation will extend their probation and may result in the loss of space in the Facility for the Principal Investigator.

V. Payment of Facility Fees:

A. Facility users who do not pay their Facility bills, risk losing their assigned space.

B.Facility users will be given 30 days notice prior to revocation of their assigned space for non-payment. In this time, PIs should either make payment in full or make arrangements for relocating their animal.

VI. Loss of Assigned Space:

Pis may lose their assigned space in the Facility for non-payment as described above or for repeated SOP violations by his/her staff/students.

A. A PI whose research program has a track record of multiple individuals being placed on probation or that has received two or more level C violations within a 12 month period will be subject to revocation of their assigned space in the Facility for the remainder of their assignment and will not be assigned space in the Facility for a period of no less than a year.

B. Decisions regarding revocation of assigned space for non-compliance to SOPs will be made by the Facility supervisor after consultation with the IACUC committee.

C. If a PI wishes to **appeal** a space revocation decision, then they should do so in writing to the ARF Supervisor within 30 days of the revocation decision.

Appendix II: Species-Specific Animal Space Requirements & Handling Procedures

Minimum space recommendations for Laboratory Animals (Guide for the Care and Use of Laboratory Animals, 2011; Eighth Edition)

MICE

A. Caging

Each mouse housed in a primary enclosure must be allotted a specific amount of floor area. This requirement will vary with the cage size and weight of the individual. The following information should be used as a guide to caging mice.

Wt. Range of Individual	Floor Area Required	Height Required
-<10 g	6 sq. in. /mouse	5 in.
10-15 g	8 sq. in. /mouse	5 in.
16-25 g	12 sq. in. /mouse	5 in.
>25 g	15 sq. in. /mouse	5 in.

Ex. If you have a cage with inside floor dimensions of $10 \times X = 6 \times 10^{-112}$ K floor space is determined by multiplying the length times the width.

10112X 6 112=68 sq. in.

If you are housing mice that weigh between 10-15 g, divide the floor area require for that weight group into the floor area of the cage.

68 sq. in. (cage) /8 sq. in. (space required/animal) = 8 Eight mice can be housed in a cage 10 mzX 6 mzX 5.

*Remember, as animals gain weight, they may have to be redistributed in order to comply with standards.

Cages available at ARF:

Small plastic cages -polycarbonate - 80 sq. in. X Small plastic cages -polycarbonate - 71 sq. in XX Small plastic cages -polycarbonate - 47 sq. in

Therefore, the housing density for mice in Small, X Small and XX Small is:

Small cage: 5 adult mice >25 g X Small cage: 4 adult mice >25 g XX Small cage: 3 adult mice >25 g

B. Environment

Light cycle: 12 hours on/ 12 hours off Humidity: 30- 70% Temperature: 68-79 degrees F (70-74 ideal)

C. Cage Cards

Each cage must be identified with a cage card. Cage cards are to be attached to the front of each cage. The following information is recommended to be on each card:

- 1. Principal Investigator's name
- 2. Strain
- 3. Protocol#
- 4. Number of animals in cage
- 5. Source

D. Handling

Mice may be easily transferred from cage to cage by gently lifting them by the base of their tails. Mice can be restrained by gently but firmly pinching the skin on the back of their necks and wrapping the tail around a finger.

E. Breeding

1. Mice reach breeding age at approximately 35-40 days of age.

2.Females may be bred either intensively (i.e., immediately after parturition) or nonintensively. **If** successive mating is desired, the mated pair should be left together, as the female has a postpartum estrus. Gestation period of the mouse is -20 days and the young are ready to be weaned at 21 days.

Polygamous mating can also be used in reproducing mice as a means to economize the number of available males. **If** breeding is to be polygamous, only one male in each breeding cage should be utilized. Adult male mice will fight and may kill one another if housed together with females.

F. Health Problems

It is the responsibility of the animal care personnel to watch for symptoms of illness or disease. Common symptoms of health problems in mice include:

- 1. Weight loss/loss of appetite
- 2. Unthrifty coat
- 3. Inactivity
- 4. Diarrhea-especially in infant mice
- 5. Labored breathing
- 6. Scabby wounds or abscesses
- 7. Lumps/masses

Record any evidence of health problems on the animal health observation sheet and report it to the Facility Supervisor immediately.

<u>RATS</u>

A. Caging

Each rat housed in a primary enclosure must be allotted a specific amount of floor area. This requirement will vary with the cage size and weight of the individual. The following information should be used as a guide to caging rats.

Wt. Range of Individual	Floor Area Required	Height Required
<100 g	17 sq. in./rat	7 in.
100-200 g	23 sq. in./rat	7 in.
200-300 g	29 sq. in./rat	7 in.
300-400 g	40 sq. in./rat	7 in.
400-500 g	60 sq. in./rat	7 in.
>500 g	70 sq. in./rat	7 in.

Eg. If you have a cage with inside floor dimensions of 25 \times 9 112, the floor space is determined by multiplying the length times the width.

25 112X 9 112= 242 sq. in.

If you are housing rats that weight between 200-300 g, divide the floor area required for that weight group into the floor area of the cage.

242 sq. in. (cage)/29 sq. in. (space required/animal) = 8

Eight rats can be housed in a cage 25 112 X 9 112 X 7.

Remember, as animals gain weight, they may have to be redistributed in order to comply with standards.

Cages available at ARF:

Medium Plastic cages -polycarbonate -214 sq. in. Large plastic cages -polycarbonate - 357 sq. in. X Large plastic cages -polycarbonate - 379 sq. in

B. Handling

Grasp the animal at the base of the tail and lift. Support the body weight at all times, do not dangle by the tail. This hold is safe for short periods of the time, and care must be taken not to damage the tail as it is easily broken and/or stripped of skin.

C. Environment Light cycle: 12 hours on/ 12 hours off Humidity: 30- 70% Temperature: 68-79 degrees F (70-74 ideal)

D. Cage Cards

Each cage must be identified with a cage card. Cage cards are to be attached to the front of each cage. The following information is recommended to be on each card:

- 1. Principal Investigator's name
- 2. Strain
- 3. Protocol #
- 4. Number of Animals in cage
- 5. Source

E. Breeding

- 1. Rats reach breeding age at approximately 70 days.
- 2. Rats are best bred non-intensively using one male for every 3 females.
- 3. The gestation period runs an average of 22 days and the young can be weaned at 21 days.

F. Health Problems

It is responsibility of all animal care personnel to watch for symptoms of illness or disease. Common symptoms of diseases in rats include:

- 1. Weight loss/loss of appetite
- 2. Unthrifty coat
- 3. Inactivity
- 4. Raised, inflamed, concentric ridges on tail
- 5. Respiratory infection

Record any evidence of health problems on the animal health observation sheet and report it to the Facility Supervisor immediately.

APPENDIX III Ponce Medical School Foundation, Inc D16-00352

Animal Welfare Assurance for Domestic Institutions

I, Kenira J. Thompson, as named Institutional Official for animal care and use at Ponce Medical School Foundation, Inc., provide assurance that this Institution will comply with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy).

I. Applicability of Assurance

This Assurance applies whenever this Institution conducts the following activities: all research, research training, experimentation, biological testing, and related activities involving live vertebrate animals supported by the PHS. This Assurance covers only those facilities and components listed below.

- A. The following are branches and components over which this Institution has legal authority, included are those that operate under a different name: All departments of Ponce Medical School Foundation, Inc. (PMSF)
- B. The following are other institution(s), or branches and components of another institution: None

II. Institutional Commitment

- A. This Institution will comply with all applicable provisions of the <u>Animal Welfare Act</u> and other Federal statutes and regulations relating to animals.
- B. This Institution is guided by the "<u>U.S. Government Principles for the Utilization and Care of</u> <u>Vertebrate Animals_ Used in Testing. Research, and Training.</u>"
- C. This Institution acknowledges and accepts responsibility for the care and use of animals involved in activities covered by this Assurance. As partial fulfillment of this responsibility, this Institution will ensure that all individuals involved in the care and use of laboratory animals understand their individual and collective responsibilities for compliance with this Assurance, and other applicable laws and regulations pertaining to animal care and use.
- *D.* This Institution has established and will maintain a program for activities involving animals according to the *Guide for the Care and Use of Laboratory Animals* (*Guide*).
- E. This Institution agrees to ensure that all performance sites engaged in activities involving live vertebrate animals under consortium (subaward) or subcontract agreements have an Animal Welfare Assurance and that the activities have Institutional Animal Care and Use Committee (IACUC) approval.

III. Institutional Program for Animal Care and Use

A. The lines of authority and responsibility for administering the program and ensuring compliance with the PHS Policy are as follows:

The institutional official responsible for administration is Dr. Kenira J. Thompson, Interim Dean of Research. The Institutional Animal Care and Use Committee (IACUC) is directly responsible to her. The supervisor of the Animal Research Facility is Ms. Lisette Laboy, who reports to Dr.

I

Kenira J. Thompson and consults with IACUC. The lines of communication are represented in the diagram below:





- B. The qualifications, authority, and percent of time contributed by the veterinarian(s) who will participate in the program are as follows:
 - 1) Name: Dr. Kenneth Thompson

Qualifications

- Degrees: DVM
- Training or experience in laboratory animal medicine or in the use of the species at the institution:

Dr. Kenneth Thompson is a contract employee as Staff veterinarian. The Veterinarian lives at 35 minutes distance from Ponce Medical School Foundation Inc. and he can be reached by telephone easily during day, night, weekend and holidays. Dr. Thompson usually devotes 5% of his time per week to the animal care and use program. He spends additional time as needed. Dr. Thompson has more than 40 years of experience in private practice and laboratory animal medicine. He is responsible for overseeing the general health of the animals and their housing, feeding, and watering conditions. At the present time he visits the animal research facility at least once a week to check on the physical condition of the animals, and is in contact with the facility administrator as required. Dr. Thompson is available at all times during working hours for advice, consultation and veterinary services regarding the health of the laboratory animals, and their proper treatment and medication administration. He participates in the design and review of the Veterinary Program, reports to the Institutional Official and consults with the IACUC. He also participates in an annual workshop that if offered to all PMSF staff and researchers involved in using animal in research. Dr. Thompson advises the IACUC and all individual researchers on all issues involving animal diagnosis, treatment and procedure of research animals, including PHS policy.

Authority: Dr. Thompson has direct program authority and responsibility for the Institution's animal care and use program including access to all animals.

In the unlikely event that Dr. Thompson is not available; Dr. Patricia Randell, a local veterinarian who has an office in Ponce, is available for emergency &/or after hours veterinary care, or for when Dr. Thompson is on vacation.

Responsibilities and Authority of Veterinarian

- a. <u>General</u> Disease control in an animal facility is based on obtaining healthy animals, maintaining them in a proper environment, prevention of cross contamination, and surveillance and treatment of diseases. This goal requires the cooperation of the investigator's staff and the Facility staff on an ongoing basis.
- b. <u>Disease Monitoring</u> Animal technicians are to report any abnormality in research animals to both the investigator and the veterinarian. When abnormalities are noted by the Investigator's staff, it is required that the animal facility staff be notified promptly.

Animals are examined upon arrival at the animal facility for general appearance and health, and the completeness and accuracy of the order is verified.

c. <u>Disease Diagnosis and Treatment</u> -Serious disease problems will require immediate attention. The Principal Investigator will be contacted before any treatment is initiated to determine what impact would have on experimental results. When such contact cannot be made the Veterinarian will defer treatment, proceed with treatment, or dispose of the animal based on humane considerations.'

Consultation by the principal investigator with the veterinarian regarding the health status of animals is expected. Conditions amenable to treatment will be promptly treated. Other conditions may not warrant treatment because of interference with experimental results or due to economic considerations.

Unexpected loss of animals not due to experimental manipulations should be discussed with the veterinarian. Necropsy and full diagnostic workup may be indicated, particularly when the condition may have the potential to become a widespread epizootic.

d. <u>Pre and Postsurgical Considerations</u> Surgical procedures will be discussed with the veterinarian when the investigator is not familiar with a procedure. Adequate anesthesia and analgesia is required.

When animals are scheduled for surgery the investigator is responsible for seeing that adequate postsurgical care is provided. There should be a written order indicating the type of postsurgical care which is to be provided, and indicating whether animal facility personnel shall provide the care, or whether the investigator's personnel will furnish the care. The care which may be provided includes oxygen, fluid, antibiotics, and analgesic. Daily observations will be made, being much more frequent during the immediate postsurgical period.

- C. The IACUC at this Institution is properly appointed according to¹PHS Policy IV.A.3.a.and is qualified through the experience and expertise of its members to oversee the Institution's animal care and use program and facilities. The IACUC consists of at 7 members, and its membership meets the composition requirements of PHS Policy IV.A.3.b. Attached is a list of the chairperson and members of the IACUC and their names, degrees, profession, titles or specialties, and institutional affiliations.
- D. The IACUC will:
 - Review at least once every 6 months the Institution's program for humane care and use of animals, using the *Guide* as a basis for evaluation. All members of the IACUC are invited to participate in the semiannual program review. The IACUC procedures for conducting semiannual program reviews are to review institutional policy and practices with respect to

providing for humane care and use of animals using the Guide as a basis for evaluation. More specifically the IACUC examines whether its membership still satisfies the requirements, and that all members are aware of the procedures for: the inspections, animal protocol reviews {and any changes or renewals to already approved protocols), investigation of animal concerns. The IACUC checks that all its records are kept up-to-date with all the minutes for meetings recorded and provided to the members, semi-annual and annual reports submitted to the 10, also that minority views are included in such reports, and that any deficiencies are described in such reports along with a plan and schedule for correction. It checks that all the information required by the OLAW is submitted in such reports promptly. It checks that records are kept from the minutes from the IACUC meetings, and that the veterinary care is as should be with records of veterinarian visits and policies for animal procedures. It checks on the animal staff training and the guidance that the veterinarian {and the IACUC itself) provides to investigators and their staff and students. It checks that all personnel are trained in health and safety, and know the correct precautions to be taken when working with animals, hygiene procedures with regard to proper clothing attire, personnel protection and eating/drinking/smoking policies. It also reviews its policies for preventive medicine, and animal receiving and transportation. It discusses the most recent recommendations on surgery procedures, anesthesia and euthanasia in order to ensure that possible pain and distress to the animals is minimized.

- 2) Inspect at least once every 6 months all of the Institution's animal facilities, (including satellite facilities) using the <u>Guide</u> as a basis for evaluation. All members of the IACUC are invited to participate in the semiannual inspection of the facilities. The IACUC procedures for conducting semiannual facility inspections are to visit all of the institution's animal research facilities and assess them for compliance with PHS requirements and standards of good practice as described in the institution's "Research Animal User's Manual" and "Manual of Standard Operating Procedures". This includes inspecting the animal housing, food, water, bedding, location, and sanitation, as well as environmental factors such as temperature, ventilation and illumination. Behavioral management, veterinary care and waste disposal procedures are also assessed. The IACUC has checklists which include a listing of all rooms (both animal rooms and the physical plant) with information on all aspects to be reviewed. The committee checks that there is separation of species, that the building itself is sound with no structural problems in the walls, windows, ceilings¹ or doors. Lighting, temperature, humidity and noise are monitored. It checks each room where the animals are housed to ensure that the temperature, ventilation and lighting are correct. It checks all the cages to look for proper sanitation, bedding, food and water, safety, size requirements, number of animals per cage, any enrichment and card information. It notes the census at the time of the inspection. It notes whether there is any environmental enrichment, it checks the location of the food and bedding storage area for proper storage, and vermin control. It checks the cage washing area. It checks records for the veterinarian visits and records of any required veterinarian care. In addition the IACUC at the time of the inspection (although often unannounced) usually has the opportunity to talk with the facility staff regarding the day-to day activities. The same procedures are followed for the satellite bio bubble facilities.
- 3) Prepare reports of the IACUC evaluations according to PHS Policy IV.B.3.and submit the reports to Dr. Kenira Thompson, the Institutional Official. The IACUC procedures for developing reports and submitting them to the Institutional Official is to incorporate the outcomes of the IACUC meetings {using the minutes} and inspections into a report prepared in the format described in IV.B.3.of the OLAW Public Health Service Policy on Humane Care and Use of Laboratory Animals. In accordance with the PHS policy the semi-annual report to the Institutional Official includes a description of the nature and extent of the Institution's adherence to the Guide for the Care and Use of Laboratory Animals (Guide). Any departures from the Guide will be identified specifically and reasons for each departure will be stated and reported to the IO for each six month reporting period during which the IACUC approved departure is in place. If there are no departures from the Guide, PHS Policy, or the Animal Welfare Act regulations, the semiannual report will state that the Institution does not have any departures. Deficiencies noted will be distinguished as minor or significant with a reasonable plan and schedule for correcting each deficiency. The reports are circulated to the IACUC members for their review and signature before submission to the Institutional Official.

Any minority views will be included with the report. The report will be signed by a majority of the IACUC members. Copies are maintained for inspection in the office of the animal research facility and in the office of the IACUC chair.

- 4) Review concerns involving the care and use of animals at the Institution. Concerns by members of the animal care staff, investigators or students can be brought to the attention of the IACUC directly (written or in person), or can be made to the veterinarian or animal house staff. This includes but is not limited to: quality of animal care, maintenance of facilities, compliance by investigators, and training of animal care technicians, investigators and laboratory technicians. In order to provide protection against reprisal, as required under the Animal Welfare Act, an anonymous 'drop-box' is located in both the animal research facility and on the ground floor of the main research building. This has been brought to the attention of faculty, students and technicians working at the institution by advertisement in animal awareness training and posted signs. Any concerns are discussed by the IACUC at one of its regular meetings and, where necessary, warnings or interventions are carried out. Recommendations are then made to the Institutional Official.
- 5) Make written recommendations to the Institutional Official regarding any aspect of the Institution's animal program, facilities, or personnel training. The Chair of the IACUC will provide a written recommendation to the Institutional Official, usually in the form of a memo, with an explanation of the issue together with the IACUC's plan or recommendation to address that issue.
- 6) Review and approve, require modifications in (to secure approval), or withhold approval of PHS-supported activities related to the care and use of animals according to PHS Policy IV.C.1-3. The IACUC procedures for protocol review are as follows: the investigator is asked to complete the PMSF Animal Subject Statement Protocol and submit an electronic copy to the IACUC. The Chair designates a reviewer for each protocol. Each protocol is circulated to all members of the IACUC asking for their concurrence with the use of a designated-member review or whether they wish to call for a full-committee review. If there is no call for a full committee review, the designated member will review the protocol, taking into consideration any comments on the protocol shared by the other committee members. The designated member will notify in writing the IACUC Chair of their decision with reasons for approval, or recommendation for modifications (to secure approval) or referral to the full committee for review. The IACUC Chair will provide a letter in writing to the Investigator outlining any modifications required, or requests for additional information. When a full-committee review is called the protocol will be discussed at a convened meeting with quorum where the members of the committee will vote to approve, require further modifications or withhold approval. Members may not participate in the review or approval of a project in which they have a conflict of interest, except to provide information, and may not contribute to the quorum for the vote on that project.
- 7) Review and approve, require modifications in (to secure approval), or withhold approval of proposed significant changes regarding the use of animals in ongoing activities according to PHS Policy IV.C. The IACUC procedures for reviewing proposed significant changes in ongoing research projects are as follows: the investigator is asked to submit a written request for any change in an ongoing research project (including but not limited to; change in number of animals, species, experimental procedures, anesthesia, animal handling or housing) to the IACUC. The Chair designates a reviewer for the requested change. A copy of the request is circulated to all members of the IACUC asking for their concurrence with the use of a designated-member review or whether they wish to call for a full-committee review. If there is no call for a full-committee review, the designated member will review the requested change, taking into consideration any comments shared by the other committee members. The designated member will notify in writing the IACUC Chair of their decision with reasons for approval, or recommendation for modifications (to secure approval) or referral to the full committee for review. The IACUC Chair will provide a letter in writing to the Investigator outlining any modifications required, or requests for additional information. When a full committee review is called the protocol will be discussed at a convened meeting with quorum where the members of the committee will vote to approve, require further modifications or

withhold approval. Members may not participate in the review or approval of a project in which they have a conflict of interest, except to provide information, and may not contribute to the quorum for the vote on that project.

- 8) Notify investigators and the Institution in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval according to PHS Policy IV.C.4. The IACUC procedures to notify investigators and the Institution of its decisions regarding protocol review are as follows: The IACUC Chair provides a written letter to the investigator of the committee's decision. Copies of all such letters are kept by the IACUC and in the office of the animal facility. Copies of the Minutes from the IACUC meetings are sent to each member of the IACUC, the Institutional Official and the President and Dean of the Institution. Their receipt must be acknowledged. If the IACUC withholds approval, the animal facility is notified immediately and the investigator cannot proceed with the use of the animals. Where modifications are requested or approval is withheld, the investigator has the opportunity to respond in writing to the committee.
- 9) Conduct continuing review of each previously approved, ongoing activity covered by PHS Policy. Each protocol is initially approved for one year from commencement of the funding of that study, and then the investigator must submit in writing a request for a renewal of approval for subsequent years. This request must state whether any changes to animal numbers, species or experimental design have taken place. This request is reviewed by the IACUC together with the original protocol using the Guide for the Care and Use of Laboratory Animals (Guide) before approval can be given. In addition investigators are sent an Evaluation of Animal Subject Usage, Welfare and Handling form for each protocol annually. This requests information to update the IACUC records on investigator contact information, animal procedures, current animal use with regard to the original projected animal numbers, how the protocol relates to the ongoing research in the investigators lab, plans for the upcoming year, and any proposed changes. Justification for the animal numbers and studies to be used in the upcoming year is required. In addition the investigator must provide information on their general future plans, and are queried about student/employee education and awareness in animal welfare. The forms are reviewed by the IACUC using the Guide for the Care and Use of Laboratory Animals (Guide) as the basis for evaluation. Should the investigator wish to continue the activities longer than three years, then a new protocol must be submitted which will undergo a de nova review as outlined under Part III. D.6
- 10) Be authorized to suspend an activity involving animals according to PHS Policy IV.C.6. The IACUC procedures for suspending an ongoing activity are as follows: The IACUC may suspend an activity that it previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the Guide, the institution's Assurance or the PHS Policy. The activity in question will be reviewed and discussed at a convened meeting of a quorum of the IACUC and a vote of a majority of the quorum present is needed for suspension of the activity. If the IACUC suspends an activity involving animals, the IO in consultation with the IACUC shall review the reasons for suspension, take the appropriate corrective action, and report that action with a full explanation to OLAW. The IACUC procedure for suspending an ongoing activity is to notify the investigator in writing the reasons for suspension of an activity and request for correction immediately. The animal facility is also notified, and the suspension is not removed until the concerns have been addressed by the investigator. A de nova review of the activity must occur by the IACUC before the suspension can be lifted. In addition, the staff veterinarian, the Supervisor of the Animal Facility or the Chairman of the IACUC may temporarily restrict any activity which in their judgment requires additional review by the IACUC.
- E. The risk-based occupational health and safety program for personnel working in laboratory animal facilities and personnel who have frequent contact with animals is as follows:

Dr. Angel Isidro (MD, Pathologist) acts as our Occupational Health Professional, together with the safety officer (Miriam Perez) they are responsible for monitoring the occupational health program. Only those persons required for the Animal Research Facility or support purposes

are authorized to enter the facility. Before entering persons are advised of any potential biohazards and are instructed on the appropriate safeguards by the area supervisor.

The animal facility has a training program that is mandatory for all individuals that are new to the facility, and must be completed prior to receiving security access to the facility and prior to beginning work in the facility. All employees working with animals are required to be familiar with and to follow the safety procedures outlined in the Institutional Research Animal User's Manual and the Manual of Standard Operating Procedures. The area supervisor together with the safety office ensures that all personnel handling animals receive training on occupational hazards, and additional training is offered to all employees via seminars and web-based activities. At the time of new employment all personnel are enrolled in the program and receive initial training according to the specific job description for the position. They then receive continuous on-the-job training on a daily basis. We also include, and enforce, the participation of the employees in the workshops and seminars that are given by the animal house and IACUC. All persons working with animals must have the standard employment physical exams, and have current tetanus prophylaxis.

Special attention is given to those personnel with specific conditions that might affect their performance, such as pregnancy, allergies, illness and/or other special conditions including decreased immunocompetence. General instructions are given to them as part of the basic information once they are hired in regard to what to do if they develop such a condition. Each case is evaluated separately and treated in accordance with the policies of "the Guide" and our Institution. The personnel are provided with personal protective equipment, and are required to have the necessary vaccines.

All incoming personnel who will be in direct or indirect contact with animals, receive proper safety training on animal handling techniques, as well as preventive measures to reduce zoonoses. Clear guidelines are in place regarding animal-related safety. In addition, they receive training on protective gear that will reduce allergy-related illnesses. Our Occupational Health Professional also offers a yearly seminar on zoonoses, available to all employees and students at Ponce Medical School Foundation, Inc.

Persons working with non-human primates should have periodic tuberculin testing or chest x-rays, as recommended by Personnel Health. Employees who work with canine and felines are encouraged to receive a pre-exposure course of rabies vaccine. At this time there are no felines, canines or non-human primates at PMSF.

Institutional policies for personal hygiene require that eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms, nor in the preparation rooms or hallways. An insect and rodent control program is also in effect.

All procedures carried out must be carefully performed to minimize the creation of aerosols or spatters. Work surfaces must be decontaminated after use or after any spill of viable material. Personnel must wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility. Institutional policies for the safe handling of sharps must be followed. Personnel are advised of special hazards, and are advised of and are required to read and follow instructions on practice and procedures.

All waste from animal rooms (including animal tissues, carcasses and contaminated bedding must be transported in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional and local requirements. Incineration is recommended and PMSF disposes waste as biomedical waste.

Hazard identification and risk assessment are routinely conducted for animal use areas and also during animal protocol and program review. A biohazards sign must be posted at the entrance to an animal room whenever infectious agents are present, The biohazard warning sign must identify the infectious agent(s) in use, list the name and telephone number of the

responsible person(s), and indicated any special requirements for entering the animal room (e.g., the need for immunizations or respirators).

Any employee bitten by an animal or injured on the job must report the incident immediately to their supervisor. Any injuries should be promptly attended to and be reported to both the supervisor and the institution's personnel office so that any necessary medical attention can be given. The individual injured is encouraged to seek medical attention even for minor injuries. They will receive first aid treatment such as cleaning of the site of injury, disinfection and bandage. All injuries require completion of accident reports within 24 hrs. of the injury. Instructions and forms are available in the Human Resources Office and the Safety Office in the PMSF Administration Building. The animal house supervisor refers the affected person to the Human Resources department where a form is completed as part of the Institutional laboratory safety program. The form is filled out and the employee is referred to the EI Fondo de Seguro de Estado or the hospital to receive any appropriate and complete additional treatment. All bites should be reported to the veterinarian and the animal isolated for observation as required. In no instance should an animal be disposed of following a bite until specific clearance has been given.

- F. The total gross number of square feet in each animal facility (including each satellite facility), the species of animals housed there and the average daily inventory of animals, by species, in each facility is provided in the attached Facility and Species Inventory table.
- G. The training or instruction available to scientists, animal technicians, and other personnel involved in animal care, treatment, or use is as follows:
 The Supervisor of the Animal Research Facility in conjunction with the staff veterinarian supervises a training and information program for all persons involved in animal care, treatment or use which includes, but is not limited to:
 - a. Seminars regarding accepted practices for care and use of animals, including PHS policy.
 - b. Videotapes and other instructional material available to all persons at this institution.
 - c. Individual instruction in general and special procedures in the care and use of animals
 - d. Training in the use of methods that minimize the numbers of animals required to obtain valid results and limit animal pain or distress
 - e. Training in the use of personnel protective equipment (eg. gloves, masks, gowns, safety glasses, head covers, shoe covers)
 - f. Training in the use of the appropriate animal housing systems
 - g. The Supervisor and IACUC offers introductory workshops to animal users and potential animal users such as incoming graduate students at least once a year through education programs at the institution. In most cases the IACUC chair provides an introductory session on the purpose, functions and importance of the IACUC and procedures to follow for potential users. In addition, -every 2-3 years the animal house together with the IACUC offers a full half day workshop incorporating hands-only training. Attendees are provided with certificates of attendance. Copies of training materials (videos of the workshops and video tutorials of animal techniques (eg, Surgery) are available in the animal house and in the IACUC chair's office for consultation.
 - h. IACUC members are provided with orientation by the IACUC chair, the animal house supervisor and the research office. They are given hard copies of the PHS policy and the guide and have access to copies of the IACUC handbook. All members are provided with a copy of the current Animal Welfare Assurance, and are regularly sent links to online training materials, resources or recent policy changes by the IACUC chair and/or secretary.

i. Online training modules available for all staff, faculty, students and IACUC members via the CITI Program.

IV. Institutional Program Evaluation and Accreditation

All of this Institution's programs and facilities (including satellite facilities) for activities involving animals have been evaluated by the IACUC within the past 6 months and will be reevaluated by the IACUC at least once every 6 months according to PHS Policy IV.B.1.-2. Reports have been and will continue to be prepared according to PHS Policy IV.B.3. All IACUC semiannual reports will include a description of the nature and extent of this Institution's adherence to the PHS Policy and the *Guide*. Any departures from the *Guide* will be identified specifically and reasons for each departure will be stated. Reports will distinguish significant deficiencies from minor deficiencies. Where program or facility deficiencies are noted, reports will contain a reasonable and specific plan and schedule *for* correcting each deficiency. Semiannual reports of the IACUC's evaluations will be submitted to the Institutional Official. Semiannual reports of IACUC evaluations will be maintained by this Institution and made available to the OLAW upon request.

This Institution is Category 2 - not accredited by the <u>Association for Assessment and Accreditation of Laboratory</u> <u>Animal Care International (AAALAC)</u>. As noted above, reports of the IACUC's semiannual evaluations (program reviews and facility inspections) will be made available upon request. The report of the most recent evaluations (program review and facility inspection) is attached.

V. Record keeping Requirements

- A. This Institution will maintain for at least 3 years:
 - 1. A copy of this Assurance and any modifications made to it, as approved by the PHS
 - 2. Minutes of IACUC meetings, including records of attendance, activities of the committee, and committee deliberations
 - 3. Records of applications, proposals, and proposed significant changes in the care and use of animals and whether IACUC approval was granted or withheld
 - 4. Records of semiannual IACUC reports and recommendations (including minority views) as forwarded to the Institutional Official, Dr. Kenira J. Thompson, President.
 - 5. Records of accrediting body determinations
- B. This Institution will maintain records that relate directly to applications, proposals, and proposed changes in ongoing activities reviewed and approved by the IAOUC for the duration of the activity and for an additional 3 years after completion of the activity.
- C. All records shall be accessible for inspection and copying by authorized OLAW or other PHS representatives at reasonable times and in a reasonable manner.

VI. Reporting Requirements

- A. The Institutional reporting period is the calendar year (January 1 December 31). The IACUC, through the Institutional Official, will submit an annual report to OLAW by January 31 of each year. The annual report will include:
 - 1. Any change in the accreditation status of the Institution (e.g., if the Institution obtains accreditation by AAALAC or AAALAC accreditation is revoked)
 - 2. Any change in the description of the Institution's program for animal care and use as described in this Assurance
 - 3. Any change in the IACUC membership
 - 4. Notification of the dates that the IACUC conducted its semiannual evaluations of the Institution's program and facilities (including satellite facilities) and submitted the evaluations to the Institutional Official, Dr. Kenira J. Thompson.
 - 5. Any minority views filed by members of the IACUC

- B. The IACUC, through the Institutional Official, will promptly provide OLAW with a full explanation of the circumstances and actions taken with respect to:
 - 1. Any serious or continuing noncompliance with the PHS Policy
 - Any serious deviations from the provisions of the *Guide* Any suspension of an activity by the IACUC
- C. Reports filed under VI.A. and VI.B. above should include any minority views filed by members of the IACUC.

VII. Institutional Endorsement and PHS Approval

A. Authorized Institutional Official				
Name: Kenira Thompson, PhD				
Title: President, Ponce Research Institute				
Name of Institution: Ponce Medical School Foundat	tion, Inc.			
Address: (street, city, state, country, postal code) PO Box 7004				
Ponce, PR 00732	Ponce, PR 00732			
Phone: 787-840-2575 x 2158	Fax: 787-841-1040			
E-mail: <u>kthompson@psm.edu</u>				
Acting officially in an authorized capacity on behalf of this Institution and with an understanding of the Institution's responsibilities under this Assurance, I assure the humane care and use of animals as soecified above.				
Signature: Kenira Thompson .,	, Date: March 17, 2017			

B. PHS Approving Official (to be completed by OLA W)

Name/Title: Office of Laboratory Animal Welfare (OLAW) National Institutes of Health 6705 Rockledge Drive RKLI, Suite 360, MSC 7982 Bethesda, MD USA 20892-7982 (FedEx Zip Code 20817) Phone: +1 (301) 496-7163 Fax: +1 (301) 451-5672

Signature:

4.

jDate:

Assurance Number:

_, _

Effective Date:

Expiration Date:

i

VIII. Membership of the IACUC

Date: March 14, 2017			
Name of Institution: Ponce Medical School Foundation, Inc.			
Assurance Number: D	016-00352		
IACUC Chairperson			
Name*: Caroline Appleyar	d		
Title*: Professor, Basic Sc	iences Dept	Degree/Cree	dentials*: PhD
Address": (street, city, stat	te, zip code)		
Department of Basic Scier PO Box 7004	nces - Physiology	Division	
Ponce, PR 00732			
E-mail*: <u>cappleyard@psm</u>	.edu		
Phone*:787-840-2575 x 2	165	Fax": 787-841-104	n
IACUC Roster		11 ax . 101-041-104	
Name of Member/	Degree/	Position Title***	PHS Policy Membership
Kenneth R Thompson		Staff Veterinarian	Veterinarian
Monica Montalvo		Private Practice	Non-affiliated member
	D. V. 191.		
James Porter	Ph.D.	Associate Professor	Scientist experienced in
			research involving animals
		Supervisor&	New eniorititie we well an
Lissette Laboy	B.S.	Animal Research Fae.	Non-scientific member
Maria Inos Suaraz	MC	Community, Choral	Non-affiliated member who
	M.S.	leader	laboratory animal user
			Scientist experienced in
Harold Saavedra	Ph.D.	Associate Professor	research involving animals
	!		
		1	

* This information is mandatory.

** Names of members, other than the chairperson and veterinarian, may be represented by a number or symbol in this submission to OLAW. Sufficient information to determine that all appointees are appropriately qualified must be provided and the identity of each member must be readily ascertainable by the institution and available to authorized OLAW or other PHS representatives upon request.

*** List specific position titles for all members, including nonaffiliated (e.g., banker, teacher, volunteer fireman; not "community member" or "retired").

**** <u>PHS Policy</u> Membership Requirements:

Veterinarian	veterinarian with training or experience in laboratory animal science and medicine or in the use of the species at the institution, who has direct or delegated program authority and responsibility for activities involving animals at the institution.
Scientist	practicing scientist experienced in research involving animals.
Nonscientist	member whose primary concerns are in a nonscientific area (e.g., ethicist, lawyer, member of the clergy).
Nonaffiliated	individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution. This member is expected to represent general community interests in the proper care and use of animals and should not be a laboratory animal user. A consulting veterinarian may not be considered nonaffiliated.

[Note: all members must be appointed by the CEO (or individual with specific written delegation to appoint members) and must be voting members. Non-voting members and alternate members must be so identified.]

· IX. Other Key Contacts (optional)

If there are other individuals within the Institution who may be contacted regarding this Assurance, please provide information below.

Contact #1	
Name:	
Title:	
Phone:	E-mail:
Contact #2	
Name:	
Title:	
Phone:	E-mail:

Facility and Species Inventory Χ.

...

Date: March 14, 2017				
Name of Institution: Ponce Medical School Foundation, Inc.				
Assurance Number:	016-00352			
Laboratory, Unit, or Buildina*	Gross Square Feet <i>linclude</i> service areas]	Species Housed [use common names, e.g., mouse, rat, rhesus. baboon. zebrafish. African clawed frog]	Approximate Average Dailv Inventory	
Animal Research Facilities*	6,886.9	Rat	15	
		Mouse	38	
Satellite Facilities I (bio bubble)	105	Rat	48	
Satellite Facilities II (bio bubble)	56	Rat	42	
Satellite Facilities III#	800	Mouse	52	
(Backup emergency satellite in case of disaster)		Rat	750	
		1		
			!	

*Gross square feet of the Animal Research Facility includes all the animal rooms, office space, storage and bathrooms.

This satellite facility is currently being used more intensively as renovations under way in the building where the main facility is located. 36

Memo

To:	Dr. Kenira Thompson
	Institutional Official for IACUC
From:	Caroline Appleyard, Ph.D.
	Chair, IACUC
Date:	16 th December 2016
Re:	Semiannual Evaluation of Animal Care and Use Program and Inspection of Facilities

This represents the semiannual report of the Institutional Animal Care and Use Committee (IACUC), as required by the PHS Policy on Humane Care and Use of Laboratory Animals and as a condition of this institution's Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare, and USDA Animal Welfare Regulations, 9 CFR Chapter I, subchapter A. as applicable.

Evaluation of the Animal Care and Use Program

The IACUC conducted its semiannual evaluation of the institution's animal care and use program on 16th November, 2016, using the *Guide for the Care and Use of Laboratory Animals (Guide)*, and, as applicable, 9 CFR Chapter I, 2.31. The extent of the institutional adherence to the aforementioned program was reviewed, as well as the current status of investigators' protocols.

No deficiencies were identified in the animal care and use program, and all aspects of the program appear to be consistent with the *P HS Policy*, the *Guide*, and applicable Animal Welfare Regulations.

Records have been maintained as required and all investigator protocols are up-to-date.

Records show that the veterinarian, Dr. Thompson, has been carrying out regular weekly

visits to the Biomedical Research Facility, and has found everything to his satisfaction.

The composition of the IACUC membership has changed. Dr. Harold Saavedra replaced Dr. Annelyn Torres-Reveron effective September $8^{1}h$, 2016.

Inspection of Animal Facilities

The IACUC inspected all animal facilities (including the bio bubbles) on 16th November 2016 using the *Guide*, and, as applicable, 9 CFR Chapter I, 2.31. The majority of the animals are housed in the 'casita' of the permanent animal facility until renovations of the main building are completed, The satellite "Bio bubbles" are located in the main research building.

A minor deficiency was noted with regard to humidity in one of the animal rooms in the main animal house and this was promptly addressed by purchase of a dehumidifier. No other deficiencies were noted in any location.

Minority Views

There were no minority views.

Signatures

Names of IACUC Members

Dr. Caroline B. Appleyard

Sra. Lissette Laboy

Dr. Maria Ines

Dr. James Porter

Dr. Monica Montalvo Dr. Kenneth Thompson

Dr. Harold Saavedra

Signatures

APPENDIX IV

TEXTOS DISPONIBLES EN FACILIDADES BIOMEDICAS

Tútulo	Autor	Catálogo
TEXBOOK OF SMALL ANIMAL SURGERY	SAUNDERS	SF SN T355 1985 V.1, V.2
MILLER'S ANATOMY OF THE DOG	EV ANS & CHRISTENSEN	
THE WORLD CONGRESS ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES: EDUCATION, RESEARCH AND TESTING	MARY ANN LIEBERT	W 20 55 AS W9227w 1995
CRIA MODERNA DE LOS CONEJOS MANUAL PRACTICO	MARIO VACCARO	
A COLOUR ATLAS OF THE RAT DISSECTION GUIDE	R.J. OLDS & J.R. OLDS	
MANUAL FOR MOSQUITO REARING AND EXPERIMENTAL TECHNIQUES	AMCA BULLETIN No. 5	
ALLERGIC SKIN DISEASES OF DOG AND CATS	REEDY/MILLER	SF 992 A44 R327a 1989
BIOSAFETY IN MICROBIOLOGICAL AND BIOMEDICAL LABORATORIES	US DEPARTMENT OF HEALTH	

THE MERCK VETERINARY MANUAL	FIFTH EDITION SIXTH EDITION	R SF 745
BAILLIERE'S COMPREHENSIVE VETERINARY DICTIONARY	BLOOD& STUDLERT	MSSSM 1986 R SF 745 <i>M555M</i> 1986
HEART RESEARCH OF ANIMALS A CRITIQUE OF ANIMALS MODELS OF CARDIOVASCULAR DISEASE	BRANDON REINES	
NECROPSY GUIDE: RODENT AND THE RABBIT	FELMAN/SEELY	
SWINE IN CARDIOVASCULAR RESEARCH	STANTIONIMERSMANN	QY 60 58
EFFECTIVE ANIMAL CARE AND USE COMMITTIES	ORLANS/SIMMONDS/ DODDS	S978 V.I,V.2
THE HUMAN USE UF ANIMALS CASE STUDIES IN ETHICAL CHOICE	ORLANS/BECUCHAMP/ DRESSER/MORTON/ GLUCH	
INTRODUCTION TO LABORATORY ANIMAL SCIENCE & TECHNOLOGY	INGLIS	QY 50 1521
ANIMAL HOSPITAL TECHNOLOGY	AMERICAN VETERINARY PUBLICATION	1980 1971

SMALL ANIMAL CLINICAL DIAGNOSIS BY LABORATORY METHODS	TVEDTENTURNWALD	R SF 711 W694S 1989
ANATOMY AND PHYSIOLOGY	FRANDSON	1974
OF FARM ANIMALS		
CLINICALTEXBOOKFOR	MCCURNIN	SF
VETERINARY TECHNICIANS		745 C641
		1990
LABORATORY ANIMAL	FOX/COHEN/	SF
MEDICINE	LOEW	996 .5 L123 1984
ANATOMY OF SMALL	POPESKOIRAJTOV Al	R
LABORATORY ANIMALS	HORAK	QY 17 C718 V.1,V. 2
SYLLABUSFORTHELABORATORY	AALAS	R
ANIMAL TECHNOLGIST		QY S984 1972
MANUAL FOR LAB. ANIMAL TECHNICIANS	AALAS	R QY 50 M2936 1984
MANUAL FOR ASSISTANT LAB. TECHNICIANS	AALAS	R QY 50 M2933 1984

LABORATORY ANIMAL

AALAS TECHNOLOGIST TRAININNG MANUAL SERIES

LABORATORY ANIMAL TECHNICIAN AALAS

ASSISTANT LABORATORY

AALAS ANIMAL TECHNICIAN

ANAESTHETIC PRINCIPLES

M.S.U.P 1981 AND TECHNIQUES

LABORATORY ANIMAL

P.A. FLECKVELL 1987 ANAESTHESIA

Appendix V: Disposal of Dead Animals

The PSMHS has a contract with the company **CONWASTE** for the disposal of dead animals or any hazardous material that is used on Campus.

Once enough material has been collected to discard, the supervisor must communicate with staff of CONW ASTE to coordinate the collection of the hazardous material.

The steps to follow to pack dangerous material are as follows:

- 1. Prepare the boxes provided by CONW ASTE with adhesive tape.
- 2. Place red bag in the box.
- 3. Place material (in black bag) inside the box which does not exceed 50 pounds.
- 4. Seal box adhesive tape and write down the following information in the box:
 - a. Date of packaging
 - b. Date collected
 - c. Name of the generator

You must be sure you have enough packing material for next time (boxes, bags and tape). If not, you should contact CONWASTE to provide you with materials to discard hazardous waste.

When the hazardous material is collected a hazardous material shipping document is signed for regulated medical waste which must be sent to PHSU Security Officer to make the processing of payment.

Appendix VI: Bedding and Food Requirements

Bedding

All animals must have access to clean cages with an absorbent bedding to maintain a comfortable dry environment. Absorbent bedding offers rats and mice the opportunity to make nests and provides hiding places. Commercially available contact bedding includes a variety of absorptive materials: hardwood chips, hardwood and softwood shavings, processed peanut hulls, and pellet corncobs.

The following bedding is used in ARF, providing a comfortable environment:

Corncobs Bedding

Pellet Paper Bedding

These type of bedding is purchased in: Agricultural Export Inc. 100 Douglass Fir Drive Apt.104 Warrington, PA 18976 US

Softwood shavings Can be bought at any local store in case of emergency.

Teklad Global 18% Rodent Diet 2018.15

Food

Rats and mice require a low fiber diet (5%) and are typically fed a commercial pellet diet referred to as "rodent chow". Mice consume approximately 5 g per 100 g of body weight in food per day, and rats consume 12 g per 100 g of body weight per day. The adequacy and availability of food can affect the health and well-being of rats and mice and can impact experimental results if the quality is inappropriate. The following rodent diet is used in ARF:

The rodent diet is purchased in:

Agricultural Export Inc. 100 Douglass Fir Drive Apt.104 Warrington, PA 18976 US

Description-2018.15 is a fixed formula non-autoclavable diet manufactured with high quality ingredients and designed to support gestation, lactation, and growth of rodents.

Appendix VII: Pest control

Insects (cockroaches and flies) and vertebrate pest species (wild rodents) can contaminate animal housing facilities and transmit diseases. The best method for controlling pests combines preventing their entry into the facility and maintaining good sanitation practices to eliminate their opportunities to live and breed.

In addition to good practice in the cleanliness of our facilities, PHSU has a contract with the company RACO Exterminating service which fumigates around the building once a month. **Never spray** in the animal rooms as this can be toxic to animals.

Information for the Company:

RACO Exterminating PO BOX 335477 Ponce, P.R. 00733-5477 (787) 840-2055 (787) 840-1979

Appendix VIII. ARF Animal Room Classification System

Policy: It is Animal Research Facilities policy to meet or exceed all federal, state, and local regulations and guidelines and comply with all institutional policies and procedures as they apply to the performance of rodent health monitoring programs. Furthermore, it is ARF policy to have one unified animal room classification system that is standardized across all divisions on the PHSU campus.

This is applicable to all faculty, staff, students and employees of the PSMHS utilizing ARF to house their research and teaching animals.

Purpose: The purpose of this policy is to ensure that all research facilities on the PHSU campus have a standardized animal room classification system designed to prevent accidental transfer of infectious agents from one room to another. All personnel involved in animal care and use must understand the animal room classification system and follow the predetermined room entry order.

Introduction: A classification scheme has been established to reflect the health status of animals in each room. ARF assigns the classification to animal rooms based on the type of experimentation and their demands and animals received from other laboratories and/ or dealer.

Procedures:

1. Animal Room Classification System

The housing systems and procedures for entering each facility are based on a graduated scale of three categories (B, C, and D) with the highest level (B) requiring the most stringent procedures. Lower levels have less rigorous operational requirements. As you progress from B to D, procedures to enter the facilities become less rigid. Individual room designations are used to determine traffic flow within and between animal facilities. If you work in multiple facilities, or have multiple rooms, it is vital that you never work with animals from a lower-level category before working with animals from a higher level category.

RODENTS:

• Level B

Barrier housed animals. Entry into a B room is prohibited if an individual has had previous contact with animals of a lower health status within the previous 24 hrs unless she/he has changed clothes.

• Level C

Barrier or conventionally housed rodents. Entry is prohibited if an individual has had previous contact with animals of the same species with a lower health status within the previous 24 hrs, unless s/he has changed clothes.

• Level D

Animals which are known to be infected with a pathogen, or have potential for exposure to pathogens. Quarantined animals are included in this classification.

Appendix IX: Bio bubble Guidelines

- 1. Do not eat or drink in the bio bubble area.
- 2. Each person should use gloves and a lab coat. The lab coat, -if cotton, should be washed regularly, or if disposable should be replaced on a regular basis.
- 3. Discard gloves and lab coats inside the bio-hazard bag.
- 4. Each person is responsible for their animals.
- 5. Don't use cell phones inside the room.
- 6. Avoid strong perfumes when you are going to work in the room.
- 7. Never inject the animals inside the room; you should use the behavior room or surgery room.
- 8. A void loud noises, like yelling, group meetings, etc.
- 9. Once the animals arrive to the Bio bubble; you should separate them as follows:
 - a. Small cages, 2 per cage (from 25gms 70gms)
 - b. Medium cages, 4 per cage (from 71gms 200gms)
 - c. Medium cages, 2 per cage (from 210gms 320gms)
- 10. Once per week, the cages, lids and bottles should be cleaned in the lab with soap and water before putting new bedding (bedding changes can be done in between as needed without having to clean the cage).
- 11. Twice a month, the cages, lids and bottles should be taken to the animal house for cleaning and replaced with new cages/lids and bottles.
- 12. Old food will be discarded (ie do not reuse if animal is sacrificed).
- 13. Do not change the timer settings.
- 14. Do not overfill the trash can with old bedding.
- 15. Clean the bench and weight balance before and after each use.

Surgery Room

- 1. Do not eat or drink in this area.
- 2. Each person should use gloves and a lab coat. The lab coat, -if cotton, should be washed regularly, or if disposable should be replaced on a regular basis.
- 3. Discard gloves and lab coats inside the bio hazard bag.
- 4. Discard syringes and needles in a plastic biohazard container.
- 5. After finishing surgeries you must clean all instruments using Alcohol 70% and Hydrogen Peroxide 3% including the stereotaxic equipment.
- 6. Animals are anesthetized using the halogenated anesthetic gas, Isoflurane or pentobarbital solution.

Safety Procedures for Use with a Vaporizer {anesthesia machine) with a Scavenging System.

A vaporizer system with a scavenging system can be used safely, if procedures are properly followed. Some systems actively remove waste anesthetic gas (WAG) from the room by direct connection to an exhaust system (tubes attach to the wall or ceiling), but these are rare outside of a surgery suite. Most portable, table top (rodent) systems rely on a carbon filter to passively scavenge.

Before Procedure:

Check vaporizer system for leaks:

With only Oxygen on, inflate bag, close pop Doff valve and check for leaks in system.

To fill vaporizer:

- Wear gloves and long sleeves to avoid skin contact.
- Wear eye protection to avoid eye exposure.
- Ideally fill with pin index or at least with funnel tip until you reach the baseline of the upper triangle.

Carbon filter (e.g. F / AIR) canister:

Carbon filters have a finite effective life span, which can be monitored by time in use, or weight.

- The weight of each new canister should be recorded before its first use. A weight increase in 50g from the original weight means the canister should be discarded and replaced with a new one.
- Before each use, the weight should be checked and recorded. If the total increase is close to 50g, it should be replaced, or monitored closely during use (weight between animals).
- To function appropriately, the carbon canister must be at a level below that of the vaporizer, to assist passive scavenging and in an upright/vertical position. Make sure the holes on the bottom of the carbon canister must not be blocked to have an adequate air flow.

During the Procedure:

Ensure the following:

- 1. The animal is receiving a proper amount of anesthesia, by constantly monitoring the animal's breathing.
- 2. All lines and valves are open and cleared.
- 3. Isoflurane level is enough to maintain the animal under anesthesia.

End of Procedure:

Before turning off the flow or disconnecting animal from circuit:

- Tum off isoflurane by closing the valve, leaving oxygen flowing.
- Allow animal to breath oxygen for a few minutes or until recovered.