

Program Description
Animal Care and Use Program

Brookhaven National Laboratory

Brookhaven Science Associates, LLC

Upton, NY 11973

For
AAALAC International

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Program Description

Section 1. Introduction

- A. State the name of the program unit and, if applicable, its parent organization. List all organizations (schools, centers, etc.) included within the program unit.

Brookhaven National Laboratory (BNL) is managed by Brookhaven Science Associates, LLC (BSA) under contract with the United States Department of Energy (DOE).

- B. Give a brief overview of the institution, its purpose and how the animal care and use program relates to the mission of the institution.

BNL is a multi-program laboratory that conducts basic and applied research in the physical, chemical, biological, materials, environmental and energy related sciences. Within this broad mission, the main area of research that uses laboratory animals is radiation biology.

- C. Note that [AAALAC International's three primary standards](#) are the *Guide for the Care and Use of Laboratory Animals (Guide)*, NRC, 2011; the *Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide)*, FASS, 2010, and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe (ETS 123). Other regulations and guidelines used (U.S. Department of Agriculture (USDA), Public Health Service (PHS) Policy, Good Laboratory Practice (GLP), Canadian Council on Animal Care (CCAC), etc.) may also apply. Describe which of the three primary standards and other regulations and guidelines are used as standards for the institutional animal care and use program and how they are applied. For example, an academic institution in the United States with an Office of Laboratory Animal Welfare (OLAW) Assurance may use the standards of the *Guide* and PHS Policy for all animals, the Animal Welfare Act regulations for covered species, and the *Ag Guide* for agricultural animals used in agricultural research and teaching (see also *Guide*, pp. 32-33). In the European Union, the standards applied might be the *Guide*, ETS 123, Directive 2010/63, and any country-specific regulations.

BNL follows the standards of the *Guide*, PHS Policy and New York State Department of Health regulations for all animals.

- D. Describe the organization and include an accurate, current, and detailed organizational chart or charts (see **Appendix 4**) detailing the lines of authority from the Institutional Official to the Attending Veterinarian, the Institutional Animal Care and Use Committee/Oversight Body (IACUC/OB), and the personnel providing animal care. Please include the title, name (*Note:* For individuals whose information is publically

available, provide the titles and names; for individuals whose information is not publicly available, you may provide titles only.), and degree (if applicable) of each individual at the level of supervisor or above. Names of animal care staff below the title of supervisor need not be included, but the titles and number of animal care personnel under each supervisor should be included. If animal care responsibility is administratively decentralized, including the management of satellite housing areas/locations, the organizational chart or charts must include all animal care programs, indicating the relationship between each administrative unit and personnel, the Attending Veterinarian, and the Institutional Official.

Robert Tribble, PhD, is the Institutional Official (IO) with overall responsibility for the Animal Care and Use Program. Implementation of the program is the responsibility of the IACUC and the Attending Veterinarian (AV), Thomas Zimmerman, DVM. The IACUC reports directly to the IO. The AV, who is contracted through the Biology Department, reports directly to the IO and to the Biology Department, which is under the direction of the Environmental, Biology, Nuclear Science and Nonproliferation (EBNN) Directorate Associate Laboratory Director Martin Schoonen. The Brookhaven Laboratory Animal Facility (BLAF) Operations Manager, MaryAnn Petry, reports to the Biology Department Chair John Shanklin or his designee. The lines of authority and responsibility for the animal care program are indicated in the organizational chart that appears in Appendix 4.

- E. Identify the key institutional representatives (including, but not limited to, the Institutional Official; IACUC/OB Chairperson; Attending Veterinarian; animal program manager; individual(s) providing biosafety, chemical hazard, and radiation safety oversight; etc.); and individuals anticipated to participate in the site visit.

R. Tribble, Institutional Official (IO)
T. Zimmerman, Attending Veterinarian (AV)
M. Schoonen, Associate Laboratory Director (ALD) for Environment, Biology, Nuclear Science, and Nonproliferation (EBNN) Directorate
J. Shanklin, Biology Department Chair
T. Green, IACUC Chair
P. Freimuth, IACUC Deputy Chair
M. Petry, Brookhaven Laboratory Animal Facility (BLAF) Operations Manager
A. Emrick, EBNN Chief Operations Officer
R. Colichio, EBNN Research Operations
J. Subramani, Occupational Medicine Division Physician
D. Mallon, IACUC Administrator
P. Genzer, Stakeholder and Community Relations
IACUC Members

- F. Briefly describe the major types of research, testing, and teaching programs involving animals and note the approximate number of principal investigators and protocols involving the use of animals. As mentioned in the [instructions](#), please complete

Appendix 5 (Animal Usage) or provide the information requested in a similar format as an Appendix.

Physiological effects (cell mutagenesis, cell aging and other cell damage, etc.) caused by proton and heavy ion radiation, such as encountered by astronauts in space.
There are approximately 35 active protocols and 30 Principal Investigators.

- G.** Note the source(s) of research funding (grants, contracts, etc.) involving the use of animals.

BNL provides institutional core support through a contract with Brookhaven Sciences Associates, LLC. The National Aeronautics and Space Administration (NASA) and the National Space Biomedical Research Institute (NSBRI) provide additional funding.

- H.** List other units (divisions, institutes, areas, departments, colleges, etc.) of your organization that house and/or use animals that are not included in this Description. If any of these are contiguous, physically or operationally (e.g., same IACUC/OB, same animal care staff), with the applicant unit, describe the association. Explain why such units are not part of this program application.

Note: Questions regarding this section should be forwarded to the AAALAC Office.

None.

- I.** Contract Facilities: If the institution contracts for animal care facilities or services for animals owned by the institution, the contractor and its AAALAC International accreditation status must be identified. If a contractor's animal care and use program is not accredited by AAALAC International, a brief description, following this Program Description outline, of the relevant contractor's programs and facilities must be provided. In addition, the species and approximate average number of animals housed in the contract facilities and the approximate distance between the institution's animal facility and the contract facility must be noted. Incorporation of the contractor program into the site visit schedule will be discussed with institutional representatives. If the institution does not contract for animal care facilities or services, so note.

None.

- J.** Note other relevant background that will assist reviewers of this report.

BNL performs work under Animal Welfare Assurance D16-00067 (Legacy A3106-01) and New York State certification A102. BNL complies with all applicable DOE requirements as stipulated in the DOE contract with BSA for the management of BNL.

Section 2. Description

I. Animal Care and Use Program

A. Program Management

1. Program Management Responsibility [Guide, pp. 13-15]

a. The Institutional Official [Guide pp. 13-14]

Describe how program needs are clearly and regularly communicated to the Institutional Official by the Attending Veterinarian, IACUC/OB, and others associated with the program.

The IO receives semi-annual reports on the animal care program and copies of IACUC meeting minutes. The IACUC Administrator reports to the IO and communicates on an informal basis of any program needs. The AV also reports to the IO. During scheduled animal NSRL runs (Spring, Summer, Fall), a summary of animal use activities, including any unusual events and current animal census, is communicated to the IO and Management through weekly reports by BLAF staff.

b. Role of the Attending Veterinarian [Guide, p. 14]

i. Describe the institutional arrangement for providing adequate veterinary care. Although individual name(s) and qualifications will be described below, identify by title the veterinarian(s) responsible for the veterinary care program, including:

- a list of responsibilities
- a description of the veterinarian's involvement in monitoring the care and use of laboratory animals
- the percentage of time devoted to supporting the animal care and use program of the institution if full-time; or the frequency and duration of visits if employed part-time or as a consultant.

Note: If preferred, this information may be provided in a Table or additional Appendix.

Thomas Zimmerman, the AV, works 4 hours per week (either on site or via email) through a contract and is responsible for the overall veterinary care, health and well-being of all laboratory animals. In conjunction with the BLAF staff, the AV performs clinical examinations and treatments, provides training for the investigative and husbandry staff regarding animal handling and procedures and provides assistance with animal procedures including surgery and necropsy. The AV also provides guidance to investigators generating new animal protocols.

- ii. List others (e.g., Principal Investigators, veterinarians serving as Principal Investigators, veterinary faculty/staff, technical staff, farm managers) who have a *direct role in the provision of veterinary care* and describe their responsibilities. The Organizational Chart(s) provided in **Appendix 4** must depict the reporting relationship between these individuals and the Attending Veterinarian.

Note: If preferred, this information may be provided in a Table or additional Appendix.

M. Petry is the BLAF Operations Manager and acts on the directions of the AV with assigned responsibilities for facility management of daily animal care and use. She is a full-time BNL employee. BLAF personnel are assigned duties by the BLAF Operations Manager. Principal Investigators provide animal care as detailed in their IACUC approved protocol.

c. Interinstitutional Collaborations [Guide, p. 15]

Describe processes for assigning animal care and use responsibility, animal ownership and IACUC/OB oversight responsibilities at off-site locations for interinstitutional collaborations.

A Memorandum of Understanding (MOU) or other document would be created that delineates the animal care and use responsibility for oversight at off-site locations. The collaborating institution's IACUC protocol may be reviewed by the BNL IACUC to ensure animal care and use procedures are in compliance with federal and local regulations.

2. Personnel Management

a. Training, Education, and Continuing Educational Opportunities

Describe *how* the IACUC/OB provides *oversight* and *evaluates the effectiveness* of training programs and the assessment of personnel competencies. Describe how training is documented.

Note: Do not include details about the training program, which should be described in the following sections.

The IACUC evaluates training program effectiveness through protocol review of personnel qualifications, i.e., years of experience, type of procedure, plus post-compliance monitoring of activities. Specific BNL training is documented in the BNL training database. Training courses are assessed periodically by Subject Matter Experts (SMEs) and incorporate any Lessons Learned from events and post-compliance monitoring issues.

All personnel identified on IACUC approved protocols are given training and instruction in the humane care and ethical use of research animals. The training program was developed to be compliant with the Animal Welfare Act, Public Health

Service Policy, and the Department of Energy policy on both computer-based modules and examinations, followed by individual hands-on training. The AV either conducts the hands-on training or certifies BLAF personnel that may also function as trainers. All training records are maintained in the Brookhaven Training Management System (BTMS) training database.

i. Veterinary and Other Professional Staff [*Guide*, pp. 15-16]

For the Attending Veterinarian and other individuals having a direct role in providing veterinary medical care (veterinarians, other professional staff listed above, private practitioners, etc.), provide: name, credentials (including degrees), and a description of their qualifications, training, and continuing education opportunities.

Note: Please do not provide curriculum vitae of personnel; if preferred, this information may be presented in a Table or additional Appendix.

Thomas Zimmerman, DVM
Attending Veterinarian
Academic Degrees: D.V.M., U. of California, Davis, 1987
M.P.V.M., U. of California, Davis, 1988
License: NY #009836
Experience and Training: Laboratory Animal Residency – 1988-1990, Diplomate American College of Laboratory Animal Medicine – 1991, 28 years of experience in laboratory animal care

MaryAnn Petry BS, CMAR, RLATG, CPIA
Operations Manager, Animal Facility
Academic Degrees: A.A.S 2002; B.S. 2003
Certifications:
AALAS Laboratory Animal Technician (LAT) 1981
AALAS Laboratory Animal Technologist (RLATG) 1984
Institute for Lab Animal Management graduate (ILAM) 1996
Certified Manager Animal Resources (CMAR) 2004
Certified Professional IACUC Administrator (CPIA) 2015
Continuing Education: Attends local and national AALAS meetings.
Experience and Training: M. Petry has more than 40 years of experience in the field of laboratory animal care and management

ii. Animal Care Personnel [*Guide*, p. 16]

1) Indicate the number of animal care personnel.

Four Full Time Equivalents (FTEs)

2) Summarize their training, certification level and type, experience, and continuing education opportunities provided.

Note: If preferred, this information may be provided in a Table or additional Appendix.

Linda Morrell- Senior Veterinary Services Assistant, ALAT certified 2013
Full-time, 12 years of experience

Deborah Snyder- Senior Veterinary Services Assistant
Full-time, 16years of experience

Corinne Baran – Laboratory Specialist
Part-time, 13 years of experience; B.A. degree

Jodi Hamilton-Senior Veterinary Services Assistant
Full-time, 13years of experience

Clarisse Laigo- Senior Veterinary Services Assistant
Full-time, 3 months experience; A.A. & A.A.S degrees

Ongoing Education/Resources: All animal facility personnel are enrolled with the AALAS Learning Library at: <http://www.aalaslearninglibrary.org/>.
AALAS certification and continuing education is supported by BNL.

iii. The Research Team [*Guide*, pp. 16-17; 115-116; 122; 124]

- 1) Describe the *general mechanisms* by which the institution or IACUC/OB ensures that research personnel have the necessary knowledge and expertise in the animal procedures proposed and the species used.

Training and experience are listed for all personnel working on an IACUC approved protocol and the PI of that protocol certifies that all individuals have appropriate expertise and training in the species being used based on approved IACUC protocols at their home institutions.

- a) Briefly describe the content of any required training.

The “Basic Overview of Laboratory Animal Care and Use” is a computer course and examination. It provides an introduction to federal, state, and local laws that govern the use of animals in research and the consequences of non-compliance. All individuals working with animals are required to take the Basic Course. Additionally, there are separate modules specific to work being performed under a protocol: Biomethodology of the Mouse, Biomethodology of the Rat, Experimental Techniques in Rodents, Post-Procedure Care of Mice and Rats: Reducing Pain and Distress, Anesthesia and Survival Surgery in Rodents. These courses are geared towards individuals with less than one year of experience. In general, animal users

have taken required training at their home institution. In addition, all personnel are required to meet with the BLAF Manager or designee for a brief orientation and tour of the BLAF prior to the start of work.

- b) Describe the timing of training requirements relative to the commencement of work.**

All required training must be in place before access is granted to the BLAF and work begins.

- c) Describe continuing education opportunities offered.**

Continuing education would be offered through the home institutions of the Principal Investigators.

- 2) Describe the process(es) to ensure surgical and related procedures are performed by qualified and trained personnel, including:**
- who determines that personnel are qualified and trained for surgical procedures
 - the roles that the Attending Veterinarian and IACUC/OB have in this determination [*Guide*, pp. 115-116]

Pre-surgical planning with the AV is used to identify training and equipment needs, and to plan post-surgical requirements to ensure appropriate monitoring. BLAF personnel work closely with investigators and would be able to ascertain if an individual required additional training with a proposed procedure.

- 3) Describe the training and experience required to perform anesthesia.** [*Guide*, p. 122]

Laboratory Animal Training - Procedural training program plus institutional monitoring and instruction by the AV.

- 4) Describe how the proficiency of personnel conducting euthanasia is ensured (especially physical methods of euthanasia).** [*Guide*, p. 124]

Laboratory Animal Training - Procedural training program plus institutional monitoring and instruction by the AV.

b. Occupational Health and Safety of Personnel [*Guide*, pp. 17-23]

i. Institutional Oversight [*Guide*, pp. 17-19]

- 1) List the institutional entities (units, departments, personnel, *etc.*) that are involved in the planning, oversight, and operation of the institutional occupational health and safety program related to animal care and use (e.g., office(s) of environmental health, institutional health services or clinics (*including contracted health services*), industrial hygienists, Institutional Biosafety Committee(s) and/or Officer(s), Radiation Safety Committee(s) and/or Officer(s).
 - Include a brief description of their responsibilities and qualifications.
 - If contracted services are used, also include their location (e.g., remote offices to which personnel must report).

The Environmental, Safety, and Health (ESH) Directorate provides field-deployed professionals and subject matter experts in all areas of environmental protection (e.g. hazardous waste, liquid effluents), radiological control (e.g. dosimetry, radiological operations), and safety and health (e.g. industrial hygiene, biosafety, industrial safety). ESH personnel are qualified in their area of expertise including certifications in Health Physics, Industrial Hygiene, and Safety Professionals.

The Occupational Medicine Clinic (OMC) Manager serves as the point-of-contact and is part of the Human Resources Directorate and works with ESH personnel, the AV and the researchers to develop and implement the Occupational Health Program. OMC is comprised of New York State certified physicians, nurses and phlebotomists. OMC operations include administrative and medical services for staff and visitors

- 2) Describe methods to identify work-related hazards and the processes used to evaluate the significance of those hazards in the context of duties and tasks. Describe both common approaches and differences, if applicable, for categories of personnel such as, but not limited to, researchers, veterinarians, husbandry staff, cage-washing staff, students, housekeeping, physical plant staff, security personnel, IACUC/OB members (including non-affiliated members), contractors, visitors, etc. [*Guide*, pp. 18-19; see also Chapters 2 and 3 in *Occupational Health and Safety in the Care and Use of Research Animals*, NRC 1997.].

Under the BNL Work Planning and Control Program, all work conducted by all staff, guests and contractors must be evaluated for potential ESH hazards and those hazards must be analyzed and addressed appropriately to prevent injury to people or damage to assets before work is performed. The program is divided into two main processes: experimental/research work and operations/routine work. The research work is approved under the Experimental Safety Review (ESR) process. Researchers propose work including procedures for handling animals exposed to or injected with hazardous materials (radiation, chemicals, etc.). Work is reviewed by field

deployed ESH staff, including consultation with Occupational Medicine if needed, and controls are put in place to mitigate the hazards, followed by approval by Line Management. Depending on the hazard, additional work permits may be required (e.g. radioactivity, biosafety). All animal users are appropriately trained and qualified before starting work to ensure the safe handling of these materials. Routine work such as animal husbandry and maintenance or repair of equipment is reviewed and approved under the Work Planning for Operations process. All hazards are identified and controls are put in place to mitigate the hazards prior to approval. All workers are trained and qualified prior to performing any work.

3) Describe methods and frequency of reassessing work-related hazards.

Work control documents are used to assess risk, establish controls and mitigate hazards. Work control documents are reviewed annually or when the scope of work changes.

4) Describe institutional programs or methods used to track and evaluate safety-related workplace incidents, including injuries, exposures, accidents, etc. Include the frequency of such assessments. [Guide, pp. 18-19]

The BNL Events and Issues Management program describes the processes used to evaluate and track safety-related workplace incidents, including injuries, exposures and accidents. The BNL Injury Management process includes all the requirements for managing a work-related injury or illness. Using a graded approach, BNL requires all events to be investigated when they occur, corrective actions developed and tracked, and lessons learned communicated. Assessment planning is based on previous assessment results/evaluations, trends in events or lessons learned.

ii. Standard Working Conditions and Baseline Precautions

The following section pertains to the Occupational Health and Safety Program for all personnel associated with the animal care and use program. Specific information regarding the use of hazardous agents is included in **subsection iii** below.

1) Medical Evaluation and Preventive Medicine for Personnel [Guide, pp. 22-23] Note: Include blank forms used for individual health assessment as Appendix 6.

- a)** Describe who (e.g., personnel assigned to job/task categories in I.A.2.b.i.2) above) receives personal medical evaluation as a component of individual risk assessment. Describe who are **not**

included and/or exempted from personal medical evaluation. *Note:* Do not include the names of personnel.

BNL employees that have contact with animals are covered by the BNL preventive medicine program. All others are covered by their home institution's program.

- b)** Describe provisions for allowing an individual to decline participation in all or parts of the medical evaluation and preventive medicine programs (if applicable). Provide an estimate (percentage) of personnel associated with the animal care and use program that have declined participation in the medical evaluation program.

BNL employees that have contact with animals are required on an annual basis to participate in the OMC's Basic Animal Researcher clinical protocol. However, they may decline any parts of the physical exam or clinical testing considered non-essential by the examining physician or NP. They are also offered a tetanus vaccination every 10 year but may also decline it. There is one staff member involved in Field Research declines the annual physical but participates in the blood testing to ensure Rabies Titers are adequate to work with wildlife.

- c)** Describe provisions for assuring confidentiality of medical information.

Employee's health records are kept strictly confidential. All who handle Personally Identifiable Information (PII) must complete the following required training courses: Information Security Awareness, Protecting Personally Identifiable Information and systems specific training required for access to DOE Privacy Act Systems of Records (SORS). Hard copies of health records are kept in a file room which is attended all day and locked at night. The OMC itself is also locked at night and under 24-7 surveillance by BNL's police group via CCTV and motion sensors. The electronic portion of the employee's record is kept in a highly secure electronic health record that is password protected, behind a secure firewall on a dedicated server, and only accessible to authorized users within the OMC to clinical staff who have signed a confidentiality agreement. All OMC computers are also password protected, with login requiring 2 factor authentication using special "HSPD-12" ID badges. Records are covered by DOE's Privacy Act, with release of information only for specified official purposes, if mandated by law or upon the written authorization of the employee.

- d)** Describe safety considerations for individuals with incidental exposure to animal care and use (e.g., contractors, personnel working in open laboratories).

A sign is posted near the entrance to the BLAF describing steps to protect from exposure to allergens. Summer students, guests, IACUC Members, maintenance workers are made aware of potential health related issues associated with the work via the BNL Work Planning and Control Processes as well as training, postings and discussion with the BLAF Manager.

- e) Describe general features of the medical evaluation and preventive medicine programs, within the context of work duties, including:
- pre-employment/pre-assignment health evaluation,
 - medical evaluations (including periodicity),
 - diagnostic tests (e.g., for tuberculosis),
 - precautions for working with potentially hazardous species (e.g., nonhuman primates, sheep, venomous species)
 - immunization programs, and
 - procedures for communicating health related issues.

For new employees who will handle animals, the Occupational Medicine program includes a pre-placement physical examination, discussion of allergens and subsequent annual examinations by a licensed physician or Nurse Practitioner. Extensive blood and urine analysis, ECG, respiratory function tests, eye and ear examinations, and blood pressure measurements are a part of the annual physical. When working with potential rabies vector animals (physical handling) interns, contractors, etc. must provide evidence of rabies vaccination. Individuals without documented proof of rabies vaccination are not allowed to physically handle rabies vector animals. Staff working with potential rabies vector animals undergo rabies vaccination and titers are routinely monitored to ensure adequacy of protection. Bites, where skin is broken, from rabies vector animals are reported to OMC and appropriate Health Officials, and the animal is retained (if possible) for testing. Any non-employees (e.g., users, guest scientists) are made aware of potential health related issues associated with the work at BNL via training and postings and discussion with the BLAF Manager. On-site medical care is available throughout BNL business days to any personnel (employees or guests). Acute illness, work-related injuries, including animal bites, etc., are immediately attended to by a medical professional. Treatment, including tetanus immunizations, may be given as necessary. If the employee is sent home, the Laboratory may require an additional examination before the employee is permitted to return to work and must be medically cleared for return to work by the OMC. Detailed records of illnesses and work-related injuries and illnesses are kept in secure files in the OMC.

- f) Describe any other entities that provide medical services (e.g., emergency care, after-hours care, special medical evaluation, contracted services). Include a brief description of their credentials

and/or qualifications, and how these entities remain knowledgeable about animal- or institution-related hazards and risks.

After hours and on weekends, BNL's on-site Emergency Medical Service (EMS) provides first aid, triage and prompt ambulance transport to nearby hospitals if needed. EMS personnel are all NYS-certified EMTs following standard, approved New York State EMS protocols. EMS is provided with the BLAF Disaster Plan to inform them of potential animal-related hazards.

2) Personnel Training Regarding Occupational Health and Safety [Guide, p. 20]

Describe general educational program(s) to inform personnel about:

- allergies,
- zoonoses,
- personal hygiene,
- physical injuries in animal facilities (e.g., noisy areas, large quantities of chemicals such as disinfectants, ergonomics) or species used (e.g., nonhuman primates, agricultural animals),
- other considerations regarding occupational health and safety.

Include in the description a summary of the topics covered, including:

- Entities responsible for providing the training
- Frequency of training or refresher training

Note: Do not include special or agent-specific training for personnel exposed to experiment-related hazardous agents; this will be provided in **Section iii.3** below.

Occupational Health and Safety information, including information on zoonoses and personal hygiene, is included in the "Basic Overview of Laboratory Animal Care and Use" computer course and examination required by all individuals who work with animals, IACUC members and associated administrative staff. New employees are also provided with handouts and meet with the Occupational Medicine physician. Annual physicals are linked to employee training. Online or classroom Hazard Communication and Rad Worker training is completed every two years. Occupational noise exposure and ergonomics is addressed through BNL's Worker Safety and Health Management System. These subject areas provide guidelines to inform personnel of physical hazards. Personal Protective Equipment (PPE) and chemical management procedures are detailed in the Laboratory's SBMS and BLAF Operations Manual. An air sampling review determined that infrequent visitors to the BLAF, including IACUC members, would not require medical

clearance since the level of allergens in the air is not sufficient to cause sensitization from a short exposure. A sign is posted near the BLAF entry to remind visitors of potential reactions to allergens and suggest use of protective equipment if necessary. Personnel are not permitted in radiologically controlled areas without proper training or escort.

3) Personal Hygiene [*Guide*, p. 20; *Ag Guide* pp. 4-5]

- a) List routine personal protective equipment and work clothing provided and/or required for animal care personnel, research and technical staff, farm employees, etc.

Technical personnel working with animals are issued disposable or cotton lab coats and gloves. BLAF personnel are provided with uniforms (shirts and pants), cotton lab coats, safety glasses or goggles (when applicable) and steel or composite toe safety shoes or safety shoes that are slip resistant. Dust masks and N-95 respirators are optional for non-hazardous work. If N-95 respirators are required, as determined by the ESR, users must be fit-tested annually.

- b) Describe arrangements for laundering work clothing.

Cotton lab coats and shirts and pants are laundered regularly by a commercial service.

- c) Describe provisions and expected practices for washing hands, showering, and changing clothes, including instances where work clothes may be worn outside the animal facility.

Clean work clothing is put on each morning and changed during the day as/if needed. BLAF personnel are required to change out of their work clothing (shirts, pants, cotton lab coats) into street clothes before they go to lunch or home for the day. Change facilities, lockers, showers, hand washing facilities and toilets for animal care personnel are located within the animal facility. Disposable lab coats are provided to guests and researchers working in animal housing areas.

- d) Describe policies regarding eating, drinking, and smoking in animal facilities.

In the BLAF, eating and drinking are permitted in the lounge room only. Eating and drinking are not permitted in any other area of the animal facility or research laboratories. Smoking is prohibited in all buildings on site.

4) Standard Personnel Protection [Guide, pp. 21-22]

- a) Describe facility design features, equipment and procedures employed to reduce potential for physical injury inherent to animal facilities (e.g., noisy areas, large quantities of chemicals such as disinfectants, ergonomics) or species used (e.g., nonhuman primates, agricultural animals).

The facility is designed so that equipment associated with animal husbandry such as cage washing and cleaning are segregated and only those who use that equipment work in that area. The least hazardous chemicals are used in cleaning and disinfecting processes and are reviewed and approved by the Safety and Health Representative as well as the Environmental Compliance Representative to mitigate environmental hazards. Dumping stations with HEPA filtration are used to limit exposures to allergens. Approved work planning documents determines what training, PPE and medical clearance is needed for a particular task and a training-needs assessment is performed for each worker to determine training requirements to perform work safely. It is reviewed when scope of work changes or, at minimum, annually. All BLAF personnel receive the following: Compressed Gas Safety (3 years), Back Safety (3 years), Regulated Medical Waste (no requalification), Radiation Safety (2 years) and Hazard Communication (2 years). To limit the potential for animal bites and scratches, web-based and hands-on training is used to instruct personnel in the proper handling and restraint of research animals. All scientific personnel undergo radiation and hazardous materials (including biohazards) training prior to initiating any experimental work as required in their approved Work Planning documents. Earmuffs are provided in the cage washing room and strobe light alarms were installed to alert BLAF personnel of fire alarms and telephone calls while the machines are running and hearing protection is worn.

- b) Describe likely sources of allergens and facility design features, equipment, and procedures employed to reduce the potential for developing Laboratory Animal Allergies (LAA).

To reduce the potential for allergen exposure, a bedding waste dump station (filtered hood with attached augur system) is used to collect and move bedding waste to an outside covered dumpster. Cage changing hoods are used when changing cages in rooms with Individually Ventilated Cages (IVC). Disposable lab coats, gloves, masks, booties, and face-shields are available for all animal users. Clean uniforms and shower facilities are available for animal care staff. Laboratory Animal Allergy (LAA) warning signs are posted at the entrances of the animal facilities.

- c) Describe likely sources of zoonoses and facility design features, equipment, and procedures employed to reduce potential exposure to zoonoses.

Receiving animals, cage cleaning, cage changing, and maintaining equipment are likely sources of exposure to zoonoses. Procedures used to reduce potential exposure include Bite/Scratch procedures, performing safety risk assessments on biological and physical hazards associated with the species, practicing good hygiene, wearing appropriate PPE, routinely monitoring vendor animal health reports, wearing work issued uniforms/shoes, and maintaining a rodent health monitoring program. Sanitation practices are monitored by the following methods: Neogen's Accupoint adenosine triphosphate (ATP) bioluminescence sanitation monitoring system is regularly used to validate effectiveness of the cleaning process by detecting microorganisms on surfaces; ATTEST Biological Monitoring System is used to regularly monitor the effectiveness of the steam sterilization cycle; vendor health reports are reviewed; random animal shipments are tape tested for pinworms. Cage dumping and changing stations have their airflow verified annually by an outside vendor.

- d) Describe the procedures for the maintenance of protective equipment and how its function is periodically assessed.

BNL's Safety Based Management System (SBMS) details procedures for maintaining PPE in the Subject Area "Personal Protective Equipment and Respirators". These guidelines are applicable to all staff, guests, and students involved in PPE use. All PPE must be checked for its integrity and used according to BNL training and manufacturer instructions. Defective PPE must not be worn and defects must be reported through BNL's Procurement and Property Management (PPM) Department. Nonconformance reports are tracked in the BNL Supplier Nonconformance (BSNC) Reporting and Tracking System. Waste bedding hood is tested, calibrated and certified annually through a service contract with ENV Services, Hatfield, PA.

- e) Respiratory Protection

- i) Describe situations where respiratory protective equipment is available or required, such as cage washing facilities, feedmills, etc.

Currently there are no tasks that require respiratory protection, however N-95 disposable masks are available near the fill and dump stations for voluntary use. BNL does have a Respiratory Protection Program should the work require it. If respiratory protective equipment use is

mandatory, training, medical clearance and fit testing would be required.

- ii) Describe programs of medical clearance, fit-testing, and training in the proper use and maintenance of respirators.

The BNL Respiratory Protection Program describes the requirements for medical clearance, training, fit testing and proper use and maintenance of respirators. If respirators are required, medical approval from a Licensed Health Care Provider must be obtained prior to use. Staff is given instruction on manufacturer's recommendations for use, fit testing and period of familiarization, handling and storing of respirators between uses, and cleaning/decontaminating procedures consistent with OSHA requirements. Guidance on respirator cartridge disposal is provided through a Waste Management or Environmental Compliance Representative.

- iii) Describe how such respiratory protective equipment is selected and its function periodically assessed.

The Respiratory Protection Subject Matter Expert is involved in the review of functions to determine if and what respiratory equipment is needed to perform the work. The ESH Rep coordinates the selection and use of appropriate respiratory equipment for work with hazardous or toxic chemicals. Procedures establish equipment selection (theory, advantages and limitations) for Face-Piece (full, half hood); type of air supply; NIOSH certification on masks, cartridges, etc. Operating parameters are assessed through factors such as service life and change out schedules for respirator cartridges.

f) Heavy Equipment and Motorized Vehicles

- i) Provide a general list of the types of cage-processing equipment used, such as rack/cage washers, tunnel washers, robotics, and bulk autoclaves. Describe training programs, informational signage, and other program policies designed to ensure personnel safety when working with such equipment.
Note: Details of specific equipment installed in animal facility(ies) are to be provided in **Appendix 15** (Facilities and Equipment for Sanitizing Materials).

Mechanical equipment such as pallet jacks are used to move food and bedding pallets and chemical drums. The BNL Material Handling Program includes the requirements for use of mechanical material handling including training, medical surveillance and job performance measures. In addition, cage and tunnel washers, carts, bedding dispenser, autoclave, and waste dump station are inspected and serviced regularly. Training for the use of the equipment are part of the animal facility's Standard Operating Procedures. Any maintenance and repair activities are reviewed and approved under the BNL Work Planning and Control program. BLAF personnel review BLAF SOP's and receive hands-on training for autoclave, cage and tunnel washer equipment operation. In addition, rack washer doors are labeled with "PUSH TO OPEN" signs for employee safety. Manufacturer manuals are also available to ensure safe equipment operation.

- ii) List other heavy equipment such as scrapers, tractors, and farm machinery (manufacturer name, model numbers, etc. are not necessary). Describe training programs, informational signage, and other program policies designed to ensure personnel safety when working with such equipment.

Note: If preferred, this information may be provided in a Table or additional Appendix.

Not applicable.

- iii) If motorized vehicles are used for animal transport, describe how the driver is protected from exposure to hazards such as allergens or zoonoses and decontamination methods employed. Also describe instances where vehicles may be shared between animal and passenger transport.

Filter top or micro-isolator lids are used to cover cages when transporting rodents in vehicles. The animal transport van is dedicated to animal transport and not used for passenger transport. When turtles are transported on site for shell notching they are typically transported in plastic containers or boxes placed in the bed of a pickup to maintain equilibrium with outdoor temperatures and isolate from the cab. Boxes are used once, and then discarded. Plastic containers are cleaned and allowed to air dry prior to re-use.

- g) Describe safety procedures for using medical gases and volatile anesthetics, including how waste anesthetic gases are scavenged.

Anesthetic gas is either vented via tubing/manifolds to the room air exhaust system or absorbed in anesthetic scavenging units (OMNICON, f-air)

attached to the anesthesia machine. The f-air filters are changed according to manufacturer's recommendations. Additional safety measures include the use of chemical fume hoods, safety glasses, gloves, lab coats, etc. The manufacturer's operating manual is followed.

iii. Animal Experimentation Involving Hazards [Guide, pp. 20-21]

- 1) List, according to each of the categories noted below, hazardous or potentially hazardous agents currently approved to be used in animals that are or will be maintained for more than a few hours following exposure. If the hazardous agent cannot be listed by name for security/proprietary reasons, identify it by the general category of agent and level of hazard. *Note:* If preferred, this information may be provided in a Table or additional Appendix.

- a) Biological agents, *noting hazard level* (CDC Biohazard Level, Directive 93/88 EEC, CDC or USDA/DHHS Select Agent, etc.). Examples may include bacteria, viruses, viral vectors, parasites, human-origin tissues, etc.

N/A

- b) Chemical agents, *noting general category* of hazard (toxicant, toxin, irritant, carcinogen, etc.). Examples may include streptozotocin, BrdU, anti-neoplastic drugs, formalin, etc.

Chemical agents that are used are reviewed by the ESH Rep and include BrDu (Bromo-deoxyuridine) – mutagen and teratogen; IdU – mutagen; Formalin ® (10% formaldehyde) – mutagen.

- c) Physical agents (radiation, UV light, magnetic fields, lasers, noise, etc.).

Ionizing radiation

- 2) **Experiment-Related Hazard Use** [Guide, pp. 18-19; See also Chapters 2 and 3 in *Occupational Health and Safety in the Care and Use of Research Animals*, NRC 1997].

Note: Written policies and standard operating procedures (SOPs) governing experimentation with hazardous biological, chemical, and physical agents should be available during the site visit.

- a) Describe the process used to identify and evaluate experimental hazards. Describe or identify the institutional entity(ies) responsible for ensuring appropriate safety review prior to study initiation.

All work is reviewed and approved under the BNL Work Planning and Control Program. Experimental work with animals that uses potentially hazardous agents must be reviewed and approved by the Experimental Safety Review (ESR) Committee and work planning documents (ESR and work permit) are required and authorization must be obtained in order to initiate any experiment utilizing hazardous agents.

- b) Describe how risks of these hazards are assessed and how procedures are developed to manage the risks. Identify the institutional entity(ies) responsible for reviewing and implementing appropriate safety or containment procedures.**

Under the BNL Work Planning and Control Program all work conducted must be evaluated for potential ESH hazards and those hazards must be analyzed and addressed appropriately to prevent injury to people or damage to assets before work is performed. The research work is approved under the Experimental Safety Review (ESR) process. Researchers propose work including procedures for handling animals exposed to or injected with hazardous materials (radiation, chemicals, etc.). Work is reviewed by field deployed ESH Reps, along with Occupational Medicine consultation, controls are put in place to mitigate the hazards and approval is given by Line Management. Depending on the hazard, additional work permits may be required (e.g. radioactivity, biosafety). All personnel have access to subject areas and subject matter that outlines guidelines for many potential safety issues. All new staff take appropriate safety training. In addition, detailed instructions are given by the local ESH Rep or line research operations staff, with emphasis on any particular issues the new employee or guest may encounter.

- c) Describe the handling, storage, method and frequency of disposal, and final disposal location for hazardous wastes, including infectious, toxic, radioactive carcasses, bedding, cages, medical sharps, and glass.**

The BNL Waste Management group manages the procedures for the handling of all regulated wastes including hazardous, radioactive and regulated medical wastes as well as any recycling or deactivation procedures. The waste that will be generated and determination of waste stream is reviewed as part of the Experimental Safety Review process. There are training courses required for generators of hazardous, radioactive and/or medical wastes. A motorized conveyor system moves the used bedding to an outside, specially designed enclosed dumpster where it is collected and sent to an on-site compost pile. Bedding known to be contaminated with infectious agents, non-radioactive animal carcasses, and sharps are disposed of as regulated medical waste (RMW). Appropriately sized, "Biohazard" labeled containers lined with a biohazard bag, are used for carcasses and contaminated bedding waste.

Sharps stored in hard plastic containers are placed in rigid, pre-labeled, and durable containers. Regulated medical waste forms and tags are used to identify waste in RMW cartons.

Radioactive waste generated is limited to short lived (<90 day half-life) isotopes and BNL personnel utilize 'Decay in Storage' procedures as per the relevant SBMS Subject Area. Frequency of disposal depends on the amount of RMW generated.

Disposable cages are used if necessary.

- d) Describe aspects of the medical evaluation and preventive health program specifically for personnel potentially exposed to hazardous agents.

Specific aspects of the health program that apply to personnel who handle animals with potential exposure to hazardous agents include:

a. Rabies vaccinations or titers, if applicable, are offered in connection with routine examinations.

b. The Radiological Control Division (RCD) closely monitors exposures to radiation and the ESH monitors for potentially toxic materials.

Thermoluminescent dosimeter badges (TLDs) are issued to all BNL employees and guests who may potentially receive radiation exposure as mandated by BNL procedures and federal rules.

Individuals working with radiological materials or in radiological areas must have up-to-date Radiation Worker training.

If a potentially hazardous condition is identified during the work planning process, employees receive additional training before work begins or additional controls are implemented to prevent or limit exposure. Employees are required to immediately report any potential hazards or exposure to their supervisor and the Occupational Medicine Clinic or appropriate division.

Personnel are informed about occupational health considerations mainly by verbal communication with the ES&H Representative, the AV, and the Occupational Medicine physicians as well as training and work planning documents. Material Safety Data Sheets (MSDS) are available on BNL's SBMS for all materials used in the animal facility such as chemicals, detergents, disinfectants, etc. To keep employees and guests informed on health topics (e.g., allergies, Lyme disease, etc.) newsletters are published periodically and seminars are held on site.

3) Hazardous Agent Training for Personnel [Guide, p. 20]

Describe special qualifications and training of staff involved with the use of hazardous agents in animals.

All personnel undergo training for the hazards identified in their Experimental Safety Review prior to initiating any experimental work. All training is documented in the BNL training database. Once training is completed, they are

qualified by their supervisor or host who provide task specific training and oversight.

4) Facilities, Equipment and Monitoring [*Guide*, pp. 19-20]

- a) Describe locations, rooms, or facilities used to house animals exposed to hazardous agents. Identify each facility according to the hazard(s) and containment levels (if appropriate).

Note: If preferred, information may be provided in a Table or additional Appendix.

N/A

- b) Describe circumstances and conditions where animals are housed in rooms outside of dedicated containment facilities (i.e., in standard animal holding rooms). Include practices and procedures used to ensure hazard containment.

N/A

- c) Describe special equipment related to hazard containment; include methods, frequency, and entity(ies) responsible for assessing proper function of such equipment.

Eyewash station within the room is maintained on a regular schedule by BNL personnel. Bump tests are performed by BLAF personnel prior to the start of work. Micro-isolator lids are used on all cages.

- d) Describe the husbandry practices in place to ensure personnel safety, including any additional personnel protective equipment used when work assignment involves hazardous agents.

If work assignments involve work with hazardous agents, job specific training is required. Work Planning and Control for Experiments and Operations procedure describes hazard controls and integrating safety in work planning. Disposable gloves, masks, shoe covers, head covers and lab coats are available for personnel protection. The need for additional protective equipment is identified prior to the start of work with hazardous agents. Room postings and work permits are developed through the work planning process and reviewed with all staff prior to entering the room. Hazard placards are posted on the room and all staff sign off on the work permit.

e) Incidental Animal Contact and Patient Areas

- i) List and describe facilities that may be used for both animal- and human-based research or patient areas, including the policies and procedures for human patient protection, facility decontamination, animal transport through common corridors or elevators, and other personnel protection procedures.

Not applicable.

- ii) Describe any *other* circumstances in which animals or caging equipment are transported in common use corridors or elevators (e.g., have the potential to come in contact with individuals not associated with the animal care and use program), and measures taken to mitigate risks associated with such use.

Animals, including those that have been exposed to hazardous agents, and caging equipment are transported when necessary from BLAF through public corridors to nearby research laboratories. Animal cages transported through public areas are covered with micro-isolator or filter-top lids and covered so animals are not visible.

B. Program Oversight

1. The Role of the IACUC/OB [*Guide*, pp. 24-40]

a. IACUC/OB Composition and Function [*Guide*, pp. 17; 24-25]

Please provide a Committee roster, indicating names, degrees, membership role, and affiliation (e.g., Department/Division) as **Appendix 7**.

- i. Describe Committee membership appointment procedures.

IACUC members are recruited from BNL employees, as well as neighboring institutions and the local community. Prospective IACUC members are interviewed by the IACUC Chair and/or the IACUC Administrator, and their credentials are reviewed by both. The IACUC Chair and Administrator then, if appropriate, recommend to the IO that the individual be appointed to the IACUC. The IO sends a letter of appointment. Members are appointed for a three year, renewable term.

- ii. Describe frequency of Committee meetings. Note that **Appendix 8** should contain the last two IACUC/OB meeting minutes.

The committee meets monthly or more often if required. Emergency meetings may be held by teleconference. Any use of telecommunications will be in accordance with NIH Notice NOT-OD-06-052 of March 24th, 2006, entitled Guidance on Use of Telecommunications for IACUC Meetings under the PHS Policy on Humane Care and Use of Laboratory Animals.

iii. Describe the orientation, training, and continuing education opportunities for IACUC/OB members. [*Guide*, p. 17]

Members are sent a letter explaining the operations of the IACUC and the following documents: the BNL/NIH Assurance, the OLAW “Public Health Service Policy on Humane Care and Use of Laboratory Animals,” the National Research Council “Guide for the Care and Use of Laboratory Animals,” the ARENA/OLAW IACUC Guidebook and the Animal Welfare Act.

The IACUC Administrator provides new members with an orientation/training session prior to their first IACUC meeting. A refresher of this training is given every three years. New IACUC members are required to take the CITI Essentials for IACUC Members. Members also take an on-line course entitled Basic Overview of Laboratory Animal Care and Use, which requires a refresher every two years.

The BNL Continuing Education policy provides new materials regarding updates on animal research policies, OLAW Commentary on Lab Animal Review columns as well as educational seminars and lectures given at BNL throughout the year.

The IACUC Administrator, Chair and IACUC members attend various national meetings throughout the year and disseminate any new information to the IACUC members and investigators as appropriate.

b. **Protocol Review** [*Guide*, pp. 25-27]

A blank copy of your institution’s protocol review form should be provided as **Appendix 9**. Also include forms used for annual renewal, modifications, amendments, etc., as applicable.

i. Describe the process for reviewing and approving animal use. Include descriptions of how:

- the IACUC/OB weighs the potential adverse effects of the study against the potential benefits that may result from the use (“harm-benefit analysis”),
- protocols that have the potential to cause pain or distress to animals are reviewed and alternative methodologies reviewed,
- veterinary input is provided, and
- the use of animals and experimental group sizes are justified.

Note: Make sure you address each of the items above.

All protocols follow the same review process regardless of funding. All protocols undergo pre-review by the AV to ensure proper anesthetic, analgesic and surgical methods are being proposed. The AV communicates with the PI if any issues arise during the review and those issues are resolved before the protocol is sent to the IACUC. The following elements are considered in the IACUC review: Hypothesis and specific aims; Adequacy of training and experience of personnel in the procedures used; Training courses required; All manipulations and experimental procedures; Whether the work duplicates previous experiments; Species and number of animals required and justification of same; Justification of species and why work can't be done in a lower phylogenetic species; Literature search for alternatives to pain/distress; Statement of how procedures have been refined to reduce pain/distress/morbidity; Food/water deprivation and/or prolonged or unusual restraint, if performed, including scientific justification; Whether death is used as an endpoint; Where animals will be housed and unusual housing and husbandry requirements including restriction of food or water; Request for scientific justification for any animals singly housed and/or not provided with environmental enrichment; List of all chemical agents used for sedation, analgesia and anesthesia including doses and route of administration; Names of individuals administering agents; Building and room numbers where agents are stored and security procedures for controlled substances; Description of surgical procedures and post-surgical monitoring; Description of anesthesia and post-surgical monitoring; Description and justification of multiple major survival surgeries; Criteria and process for timely interventions, removal of animals from a study, or euthanasia if painful or stressful outcomes are encountered; Method of euthanasia or disposition of animal; Safety of working environment for personnel; PI's assurance that the work does not involve unnecessary duplication of efforts; Shipping procedures including names of accepted vendors, if applicable; IACUC approval from other institutions, if so required.

- ii. Describe the process for reviewing and approving amendments, modifications, and revised protocols. If applicable, include a description/definition of "major" vs. "minor" amendments.
Note: If preferred, this information may be provided in a Table or additional Appendix.

Amendments are reviewed either at a Full Board Meeting, by Designated Member Review (DMR) or by Veterinary Verification and Consultation (VVC). For DMR, the IACUC Chair and Attending Vet determine whether DMR is acceptable based on the type of change being proposed (i.e., major or minor). Major addenda are reviewed at a Full Board Meeting and include, but are not limited to, a change in scope or PI of the protocol, addition of a surgical procedure, any change that would increase pain/distress to an animal, addition of a new species or addition of food or water restriction. For DMR, the amendment is sent to all committee members who have a time period, usually two days, in which to request that the amendment be reviewed at a full board meeting. If there are no such requests, the IACUC Chair

and AV perform the DMR. For VVC, the IACUC Chair and Attending Vet determine whether VVC is acceptable based on the type of change being proposed. Changes permitted to receive VVC are changes in animal vendor, strain, age, sex, change in anesthesia from/to ketamine/xylazine or isoflurane, change in analgesics from/to ketorolac/ketoprofen/buprenorphine, change in euthanasia method from/to CO2/cervical dislocation/euthanasia solution injection, change in identification method from/to ear tag/ear punch/ tail or paw tattoo, addition of animal euthanasia at BNL, addition of blood or tissue sample timepoints at BNL, addition of animals and change in ion, energy and/or dose rate (Consultation with NSRL Liaison Biologist and/or NSRL PI required) and increase in animal numbers. All IACUC members are informed of amendments approved through the subsequent IACUC minutes.

c. Special Considerations for IACUC/OB Review [*Guide*, pp. 5; 27-33]

i. Experimental and Humane Endpoints [*Guide*, pp. 27-28]

- 1) Describe the IACUC/OB's review of "humane endpoints," i.e., alternatives to experimental endpoints to prevent or in response to unrelieved animal pain and distress.

All protocol applications include information on the potential adverse effects that may be encountered based on the procedures being performed, and what treatments would be implemented should these effects occur. These are evaluated by the IACUC and AV during the protocol review process. Also described in the protocol are the monitoring frequency and clinical parameters to be monitored for example appearance, activity, movement, urine/feces, grooming, weight loss, vocalization, posture, etc. Finally, the protocol lists conditions or complications that would lead to the removal of the animal from the study with subsequent euthanasia. Investigators are required to perform a literature search for alternatives to pain/distress in the initial, annual and triennial applications.

- 2) For studies in which humane alternative endpoints are not available, describe the IACUC/OB's consideration of animal monitoring and other means used to minimize pain and distress (e.g., pilot studies, special monitoring, other alternatives).

Not applicable.

- 3) Identify personnel responsible for monitoring animals for potential pain and distress and describe any mechanisms in place to ensure that the personnel have received appropriate species- and study-specific training.

PIs, research technicians and BLAF personnel are responsible for monitoring animals. Training for all researchers on pain/distress is certified by the PI in the protocol. Attending Vet is available for additional training based on protocol requirements. BLAF personnel participate in online AALAS webinars to enhance knowledge of potential pain and distress in animals. BLAF training webinars – ulcerative dermatitis, specific courses in AAALAS learning library

ii. Unexpected Outcomes that Affect Animal Well-being [*Guide*, pp. 28-29]

Describe how unexpected outcomes of experimental procedures (e.g., unexpected morbidity or mortality, unanticipated phenotypes in genetically-modified animals) are identified, interpreted, and reported to the IACUC/OB.

The reporting of any unanticipated adverse effects is required during the protocol annual review, or immediately if serious. A description of what occurred is evaluated by the IACUC and any recommendations they may have for preventing future occurrences is provided to the investigator.

In addition, all animals are examined daily by the animal care staff and any abnormalities are reported immediately to the supervisor and AV for evaluation and treatment, if warranted.

The AV reports any recurring issues to the IACUC.

iii. Physical Restraint [*Guide*, pp. 29-30]

Note: This section is to include only those protocols that require prolonged restraint. Brief restraint for the purpose of performing routine clinical or experimental procedures need not be described.

- 1) Briefly describe the policies for the use of physical restraint procedures or devices. Include, if applicable, the IACUC/OB definition of “prolonged.”

Any investigator who requires the use of physical restraint in their research project must include a complete description of the restraint method, including any acclimation; how the animal will be monitored when restrained; and how the need for restraint is scientifically justified. Restraint procedures are approved as part of the routine IACUC review process. The definition of “prolonged” would be anything longer than brief restraint for the purpose of performing routine clinical or experimental procedures.

- 2) Describe animal restraint devices that are used or have been used within the last three years. For each device, briefly describe

- the duration of confinement
- acclimation procedures
- monitoring procedures
- criteria for removing animals that do not adapt or acclimate, and

- provision of veterinary care for animals with adverse clinical consequences.

Note: If preferred, this information may be provided in a Table or additional Appendix.

Hind Limb Unloading. Rodents are suspended by tail. Animals generally acclimate within three to five days for both the tail and body suspension protocols. Tail suspension animals are monitored at least twice a day for signs of stress. Weight loss, general appearance and activity levels provide a sensitive indicator of how well an animal accommodates to irradiation as well as hindlimb unloading. An average weight loss of more than 15% in any animal would be considered excessive and the animal would be removed from the study and euthanized. Endpoint criteria include reduced movement, altered behavior, eating/drinking and negative interactions with cage-mates. Rats and mice are restrained in Lucite holders for time periods from 15 minutes up to several hours. When inside the tubes, the animals will be monitored for vital signs such as respiration and movement. Animals in Lucite holders may be given food/water and monitored via camera. Video monitors were recently installed in NSRL animal holding rooms for increased viewing capability during rodent irradiation exposures. Veterinary care is available for all animals that show adverse clinical effects.

iv. Multiple Survival Surgical Procedures [Guide, p. 30]

Note: One survival surgical procedure followed by a non-survival procedure is not included in this category.

- 1) Describe the IACUC/OB's expectations regarding multiple survival surgery (major or minor) on a single animal.

Multiple major survival surgeries on a single animal are allowed provided they are scientifically justified, included in IACUC protocol and that protocol is approved by the BNL IACUC. Protocols involving multiple major survival surgeries are reviewed by the full IACUC at a convened meeting. If the IACUC believes appropriate surgical techniques are employed and if appropriate aftercare, pain relief, and follow up monitoring will occur, the procedures may be approved.

- 2) Summarize the types of protocols currently approved that involve multiple major survival surgical procedures

Note: If preferred, this information may be provided in a Table or additional Appendix.

Multiple major survival surgeries are not currently performed at BNL.

- v. **Food and Fluid Regulation** [*Guide*, pp. 30-31]. *Note:* This does not include pre-surgical fast.

Summarize the types of protocols that require food and/or fluid regulation or restriction, including:

- justification
- species involved
- length and type of food/fluid regulation
- animal health monitoring procedures and frequency (e.g., body weight, blood urea nitrogen, urine/fecal output, food/fluid consumption)
- methods of ensuring adequate nutrition and hydration during the regulated period

Note: If preferred, this information may be provided in a Table or additional Appendix.

One protocol restricts food for rats . They will be fed anywhere between 10-20 grams of food per days to maintain their weights (males: ~335-350 grams; females: ~225-250 grams). Rats are trained using operant conditioning procedures to press response levers, nose poke keys, or computer touch screens in experimental chambers, and are reinforced with delivery of 45-mg food pellets for correct responses. Typically, rats receive around 70 to 80 percent of their food in the behavioral sessions and are then supplemented with measured amounts of rat chow (e.g., 4–7 g) after the experimental session to maintain their weights at approximately 350 g or 250 g \pm 5 g. Extra rat chow is provided on weekends, or when no experimental sessions occur.

- vi. **Use of Non-Pharmaceutical-Grade Drugs and Other Substances** [*Guide*, p. 31]

Describe the IACUC/OB's expectations regarding the justification for using non-pharmaceutical-grade drugs or other substances, if applicable.

There are some studies that require drugs or compounds that are not available in pharmaceutical grade formulations. Pilot studies may also be required to determine the appropriate dose levels. Use of these drugs must be scientifically justified and their preparation must follow sterile technique.

- vii. **Field Investigations** [*Guide*, p. 32]

Describe any additional considerations used by the IACUC/OB when reviewing field investigations of animals (non-domesticated vertebrate species), if applicable.

Field research studies that involve contact with vertebrate animals are covered by an IACUC approved protocol. Additional considerations would be addressed based on species of animal used.

viii. Animal Reuse [*Guide*, p. 5]

- 1) Describe institutional policies regarding, and oversight of, animal reuse (i.e., on multiple teaching or research protocols).

The IACUC protocol requests information about the disposition of the animal at the end of the study. If the study does not include invasive procedures (i.e., surgery), non-pharmaceutical compounds or unrelieved pain/distress, the animal may be transferred to another approved protocol. The AV or BLAF Manager reviews all transfer requests to ensure the animal's history does not preclude reuse. Investigators are notified by e-mail if animals are available for use and/or reuse.

- 2) Briefly describe the types of activities currently approved that involve the reuse of individual animals.

Note: A list of specific protocols involving reuse of animals should be available during the site visit.

None.

- 3) Describe other instances where the final disposition of animals following study does not involve euthanasia, including adoption, re-homing, rehabilitation, etc.

Note: A list of specific protocols involving reuse of animals should be available during the site visit.

None.

2. Post-Approval Monitoring [*Guide*, pp. 33-34]

- a. Describe mechanisms for IACUC/OB review of ongoing studies and periodic proposal/protocol reviews (e.g., annual, biennial, triennial, or other frequency).

The approval period is determined by the IACUC during a Full Committee Review and shall be for a period of time that shall not exceed one year, but which may be of shorter duration either at the request of the PI or as a result of other concerns arising during protocol review which mandate a shortened approval period. All protocols must be submitted as a new application every three years.

- b. Describe the process and frequency with which the IACUC/OB reviews the program of animal care and use.

Every six months, the IACUC reviews the AAALAC Program Description for Animal Care and Use.

A copy of the Program is reviewed by the IACUC using the “Semi-Annual Program Review Checklist” from Office of Laboratory Animal Welfare (OLAW). Individual members are assigned sections of the Program on a rotating basis.

All animal-related SOPs, including the Disaster Plan and the IACUC policies and procedures, are reviewed at least every three years.

Any comments or changes recommended and approved by the IACUC are made to the Program following the meeting at which the Program was reviewed.

A Semi-Annual Report draft is prepared by the IACUC Administrator based on findings from the IACUC semi-annual facility inspections. Findings must be corrected by the required date and noted in the report. The Semi-Annual Report includes any other information about which the IACUC wants to apprise the Institutional Official, including the animal census for the previous six (6) months. The draft report is sent to the IACUC as part of the next agenda for review and approval.

The Semi-Annual Report is reviewed and approved by the IACUC at the next IACUC meeting. All IACUC members present sign the signature page.

The Semi-Annual Report is sent to the Institutional Official.

- c. Describe the process and frequency with which the IACUC/OB conducts facility and laboratory inspections.
- Describe the rationale or criteria used for exempting or varying the frequency of reviewing satellite holding facilities and/or animal use areas.
 - If contract facilities or contractor-provided personnel are used, describe procedures used by the IACUC/OB to review such programs and facilities.
- Note:* A copy of the last report of these reviews should be included as **Appendix 10**.

Every six months, the IACUC inspects the BLAF in Building 490, the NASA Space Radiation Laboratory (NSRL) animal holding area in Building 958, and vehicles used for animal transport. The facility inspections are conducted using the “Facility Inspection Checklist” from OLAW. Previous findings are reviewed to determine if there are ongoing findings/issues.

A subcommittee of the IACUC consisting of at least two members shall inspect the laboratories where experiments involving animals are conducted. An alternate member cannot be counted as a member of the subcommittee if their regular member is on the same inspection.

For post-approval monitoring, two protocols are selected and required training for those personnel is checked in the BNL database and the Experimental Safety Review (ESR) is compared to ensure the protocol contains the same information as the ESR. If

experiments are being performed during the walk-through, personnel are questioned to ensure the protocol is being followed.

- d. If applicable, summarize deficiencies noted during external regulatory inspections within the past three years (e.g., funding agencies, government, or other regulatory agencies) and describe institutional responses to those deficiencies. *Note:* Copies of all such inspection reports (if available) should be available for review by the site visitors.

Over the past three years, the USDA has inspected the BNL facilities two times. There were no findings.

- e. Describe any other monitoring mechanisms or procedures used to facilitate ongoing protocol assessment and compliance, if applicable.

Inspections by the New York State Department of Health are conducted annually and internal safety walk-throughs are performed by the safety department on a quarterly basis. There have been no findings reported.

3. Investigating and Reporting Animal Welfare Concerns [*Guide*, pp. 23-24]
Describe institutional methods for reporting and investigating animal welfare concerns.

Reports of concerns involving the care and use of animals are submitted in writing or verbally to the Institutional Official, IACUC Chair and/or AV. Reports may be submitted anonymously. The IACUC Chair or designee will thoroughly investigate all aspects of the allegation and prepare a written incident report. The incident report will be submitted to the IACUC for review and discussion.

The IACUC Chair will send a copy of the incident report and a memo outlining the alleged noncompliance issues to the Principal Investigators involved inviting them to appear at an IACUC meeting if the investigation supported the validity of the allegations.

The IACUC Chair will send a memo to the Institutional Official (IO) delineating the noncompliance issues and the recommended corrective actions/sanctions. The Institutional Official, in consultation with the IACUC Chair and AV, may impose further corrective actions/sanctions for the investigator or support the IACUC's recommended corrective actions/sanctions.

The investigator is sent a letter describing the noncompliance issues and the required corrective actions/sanctions, with related deadlines, prescribed by the IACUC. The letter will also inform the investigator of his/her option to appeal the decision by providing to the IACUC Chair, within a specified number of days of receipt of the letter a memorandum detailing the basis of the appeal and requesting a second meeting with the IACUC.

The originator of the allegation is informed, if necessary, of the IACUC's disposition of the allegation.

Serious or continuing non-compliance is reported as required by federal policy. Additionally, BNL has an institutional Anti-Retaliation policy.

4. Disaster Planning and Emergency Preparedness [Guide p. 35]

Briefly describe the plan for responding to a disaster potentially impacting the animal care and use program:

- Identify those institutional components and personnel which would participate in the response.
- Briefly describe provisions for addressing animal needs and minimizing impact to animal welfare.

Note: A copy of disaster plan(s) impacting the animal care and use program must be available for review by the site visitors.

The care, handling and health of animals are the primary goal of BLAF personnel. The staff is responsible for providing daily care to the research animals and taking appropriate measures to protect the animals' living environment. A continuous supply of food that meets a specified nutrition formula and a continuous supply of potable water are required to ensure the safety and health of the animals at the facility. Environmental support is dependent upon continuous electric power and a correctly functioning HVAC system. For emergency power outages, there is a back-up generator for the animal facility. In the event of an emergency situation, researchers will be contacted as soon as possible. They will be apprised of the condition of their animals, power outages and room temperature changes. If contact with the investigator is not possible, the veterinarian or facility manager will decide what conditions are appropriate for the species and the ongoing experiment. The regular animal care staff that normally provide weekend and holiday coverage shall make every attempt to provide animal care in an emergency situation. The BLAF Manager, AV and BLAF personnel are accessible by phone and are listed as Emergency Personnel in the event of a disaster and permitted onsite during any BNL closures.

II. Animal Environment, Housing and Management

Note: Complete each section including, where applicable, procedures performed in farm settings, field studies, aquatic environments, etc.

A. Animal Environment

Note: Facility-specific details regarding mechanical system construction and operation is requested in Section IV.B.5. and **Appendix 11**; current (measured **within the last 12 months**), detailed (by room) performance data must also be provided as indicated in **Appendix 11**.

1. Temperature and Humidity [Guide, pp. 43-45]

- a. Describe the methods and frequencies of assessing, monitoring, and documenting that animal room or housing area temperature and humidity is appropriate for each species.

Note: If preferred, this information may be provided in a Table or additional

Appendix.

All animal rooms at the BLAF and NSRL are monitored continuously by an “Automated Logic Control” (ALC) building management system. The NSRL animal rooms A 1, 2, and 3 are monitored by ALC and individual room hygrometers. Hygrometer readings are documented by the BLAF personnel.

- b. List, by species, set-points and daily fluctuations considered acceptable for animal holding room temperature and relative humidity.

Note: If preferred, this information may be provided in a Table or additional Appendix. [*Guide*, pp. 44 and 139-140]

For rodents, temperature is set at 72 degrees F, plus or minus 2; RH ranges between 30-70% with an optimal set point of 50%.

- c. Temperature set-points in animal housing rooms and/or environmental conditions are often outside of the species-specific thermoneutral zone. Describe the process for enabling behavioral thermoregulation (e.g., nesting material, shelter, etc.) or other means used to ensure that animals can control their thermoregulatory environment. Include a description of IACUC/OB approved exceptions, if applicable. [*Guide*, p. 43]

Room temperature settings are maintained between 70-74 degrees F which is within the range of 68-79 degrees F recommended by the “Guide”, p. 44. To avoid cold-stress and allow for thermoregulation, rodents are provided with nesting material and microisolator covers. For hind-limb unloaded studies, room temperatures are set between 74-78 degrees F, plus or minus 2.

2. Ventilation and Air Quality [*Guide*, pp. 45-47]

- a. Describe the methods and frequencies of assessing, monitoring, and documenting the animal room ventilation rates and pressure gradients (with respect to adjacent areas).

Note: If preferred, this information may be provided in a Table or additional Appendix.

The EBNN Directorate Research Operations individual, using appropriate airflow measuring equipment, measures ventilation rates and pressure gradients annually in Bldg. 490. Documentation is provided on the attached HVAC System Summary. NSRL (Building 958):

Rooms A1, A2 and A3 are supplied by a single HVAC system providing a once-through air at the rate of approximately 12 changes per hour. The system is continuously monitored by the BNL Automated Logic System. Air pressure in Rooms A1 and A2 are slightly positive with respect to the Animal Area hallway – air pressure in Room A3 is negative with respect to the Animal Area hallway.

- b. Describe ventilation aspects of any special primary enclosures using forced ventilation.

Ventilated cage racks are used for rodents in Bldgs. 490 and 958 Non-recirculated room air is drawn into a filtered positive blower system then delivered to the animal cage through two grommets. Return air flows through the cage and is drawn through the filtered cage top into a plenum by the negative blower system, then into HEPA and carbon filters before exhausting into the room.

- c. If any supply air used in a room or primary enclosure is [recycled](#), describe the percent and source of the air and how gaseous and particulate contaminants are removed.

Not applicable.

3. Life Support Systems for Aquatic Species [Guide, pp. 84-87]

- a. Provide a general description of institutional requirements for enclosures using water as the primary environmental medium for a species (e.g., aquatics).

Not applicable.

- b. Provide a general description of overall system(s) design, housing densities, and water treatment, maintenance, and quality assurance that are used to ensure species appropriateness.

Note: Facility-specific tank design and parameter monitoring frequencies should be summarized in **Appendix 12** (Aquatic Systems Summary).

Not applicable.

4. Noise and [Vibration](#) [Guide, pp. 49-50]

Describe facility design features and other methods used to control, reduce, or prevent excessive noise and vibration in the animal facility.

Animal housing areas are separated from utility areas. Cinderblock construction and steel doors dampen excessive noise and vibration. All other background noise is kept to a minimum.

B. Animal Housing (all terrestrial, flighted, and aquatic species)

1. Primary Enclosures

Note: A description of primary enclosures used (e.g., cages (conventional, individually-ventilated cage systems (IVCS), etc.), pens, stalls, pastures, aviaries,

tanks) should be included in **Appendix 13**.

- a. Describe considerations, performance criteria and guiding documents (e.g. *Guide*, *Ag Guide*, ETS 123 and/or other applicable standards) used by the IACUC/OB to verify adequacy of space provided for all research animals, including traditional laboratory animal species, agricultural animals, aquatic species, and wildlife when reviewing biomedical, field and agricultural research studies.

The Guide is the standard used by the IACUC to verify the adequacy of the space provided for all research species.

- b. Describe space [exceptions](#) to the guiding documents (*Guide*, *Ag Guide*, ETS 123, and/or applicable standards), indicating the references, considerations and performance criteria used (e.g., by the IACUC/OB) to verify adequacy of space provided for all animal species covered by the program. [*Guide*, pp. 55-63]

Exceptions must be scientifically justified by the investigator and approved by the IACUC. There are currently no exceptions to the guidelines.

2. Environmental Enrichment, Social, and Behavioral Management [*Guide*, pp. 52-55; 63-65: *Ag Guide*, Chapter 4]

a. Environmental Enrichment

- i. Describe the structural elements of the environment of primary enclosures that may enhance the well-being of animals housed (e.g., resting boards, privacy areas, shelves/perches, swings, hammocks).

N/A

- ii. Describe nonstructural provisions to encourage animals to exhibit species typical activity patterns (e.g., exercise, gnawing, access to pens, opportunity for exploration, control over environment, foraging, denning, burrowing, nesting materials, toys/manipulanda, browsing, grazing, rooting, climbing).

Rats that must be singly housed will be given a nylabone® and mice will be given a Nestlet® and a Shepherd shack or tunnel, or other environmental enrichment device deemed appropriate by the AV, in consultation with the investigator.

b. Social Environment [*Guide*, p. 64]

- i. Describe institutional expectations or strategies for [social housing](#) of animals.

Unless scientifically justified, same sex animals shall be housed in compatible groups. Interim approval for exceptions to this policy can be granted by the AV, pending review and full approval by the IACUC.

- ii. Describe exceptions to these expectations (e.g., veterinary care, social incompatibility) and other typical justification approved by the IACUC/OB for housing animals individually.

If rodents fight, they are housed separately but given additional enrichment.

- iii. Describe steps taken with isolated or individually housed animals to compensate for the absence of other animals (interaction with humans, environmental enrichment, etc.).

Additional environmental enrichment such as nestlets and nylabones and a Shepherd shack or tunnel are given to singly housed mice and rats.

c. Enrichment, Social and Behavioral Management Program Review [*Guide*, pp. 58, 69]

Describe how enrichment programs and exceptions to social housing of social species are regularly reviewed to ensure that they are beneficial to animal well-being and consistent with the goals of animal use.

Enrichment programs are reviewed triennially and exceptions to the program are reviewed by the IACUC at least once a year.

d. Procedural Habituation and Training of Animals [*Guide*, pp. 64-65]

Describe how animals are habituated to routine husbandry or experimental procedures, when possible, to assist animals to better cope with their environment by reducing stress associated with novel procedures or people.

Rodents are acclimated upon arrival for at least 24 hours and preferably longer before being used for any study.

Rodents being used on specific behavioral protocols are typically acclimated to the testing apparatus prior to data collection. Animals used for Hind-limb unloading studies are acclimated to the experimental device several days prior to the study.

e. Sheltered or Outdoor Housing [*Guide*, pp. 54-55]

- i. Describe the environment (e.g., barn, corral, pasture, field enclosure, flight cage, pond, or island).

Not applicable.

- ii. Describe methods used to protect animals from weather extremes, predators, and escape (windbreaks, shelters, shaded areas, areas with forced ventilation, heat radiating structures, access to conditioned spaces, etc.).

Not applicable.

- iii. Describe protective or escape mechanisms for submissive animals, how access to food and water is assured, provisions for enrichment, and efforts to group compatible animals.

Not applicable.

f. Naturalistic Environments [*Guide*, p. 55]

- i. Describe types of naturalistic environments (forests, islands) and how animals are monitored for animal well-being (e.g., overall health, protection from predation).

Field studies are conducted with reptiles, amphibians, fish, birds and mammals on BNL grounds.

For live trapped animals: Small mammal traps are typically set overnight and must be checked within 24 hours but are routinely checked first thing the following morning. Traps are baited with high energy foods (peanut butter and oats) and nesting materials are provided to allow small mammals something to shred. In colder seasons traps are provided with wool nesting material for increased warmth. Larger mammals may be trapped using box traps or soft hold leg-hold traps. Traps are checked daily.

Fish and amphibians: Typically, they are captured, sexed, weighed and measured, then released.

Birds/bats: If mist netting is used, nets are monitored every 10 minutes.

- ii. Describe how food, water, and shelter are provided.

Other than bait utilized for trapping purposes, field investigations do not provide supplemental food, water, or shelter.

- iii. Describe how animals are captured.

Small mammal trapping is done only when weather conditions warrant or bedding is placed in traps. Traps are baited with high energy (peanut butter/rolled oats) bait. Traps are set over night, checked first thing in morning, and closed for the day.

When electrofishing is conducted, voltage and amperage are adjusted for fish size and species being targeted for capture. Depending on water body (depth, water

clarity, vegetation present) electroshocking may be used if warranted. In cases where electroshocking can't be used, seine nets, hoop nets or dip nets are used. For flying squirrels in the field, Sherman box traps are mounted on a tree about 1.5 meters high using a modified c-clamp. Traps are opened at night and baited with a peanut butter/rolled oats mixed bait. Each morning traps are checked and closed for the day. Any non-target animals will be checked for injury, noted and released. Box Traps or Leg hold traps are used for raccoons, foxes, skunks, opossum and feral cats. Traps are sized for the appropriate species. If target or non-target species are injured during trapping, they are taken to a local emergency Veterinary hospital.

C. Animal Facility Management

1. Husbandry

a. Food [*Guide*, pp. 65-67]

i. List type and source of food stuffs.

Food is manufactured by Ralston Purina Company and is delivered by WF Fisher & Sons, Somerville, NJ. The following foodstuffs are used:
Purina Mouse Chow
Purina Rodent Lab Chow
Purina Pico Rodent & Mouse Lab Chows
Envigo Laboratory, Somerset N.J. produces and distributes Teklad Rodent Chow
Research protocols may require special diets that are reviewed and approved by the IACUC before use.

ii. Describe feed storage facilities, noting temperature, relative humidity, and vermin control measures, and container (e.g., bag) handling practices, for each of the following:

- vendors (if more than one source, describe each)
- centralized or bulk food storage facilities if applicable
- animal facility or vivarium feed storage rooms
- storage containers within animal holding rooms

WF Fisher & Sons, Somerville, NJ vends all Purina food. Food is stored by the vendor on pallets in a vermin-controlled room that is kept at ~60°F. Food is never stored for longer than 2 weeks at the vendor's facility. W.F. Fisher & Sons is an "ISO" certified facility. Envigo Laboratory, Somerset N.J. supplies Teklad Diet for rodents, as requested by the research investigator.
All animal food in the BLAF is stored on mobile carts in a room that is kept at or below 70°F and ~50% humidity. The room is solid block concrete construction,

with impervious ceiling, epoxy painted concrete floor and doors are fitted with door sweeps to ensure that it remains vermin free. In addition, sticky traps are placed near the doors and are monitored daily and documented by the BLAF personnel when changed.

Food is stored in ergonomic feed bins with close-fitting, vermin-proof lids. A food label is affixed to each food bin that describes its contents, food mill date, expiration date, and date that the bin was cleaned. Food containers are sanitized at a minimum of once per month.

- iii. Describe special food preparation areas, such as feedmills and locations where special diets are formulated, if applicable. Include in the description sanitation and personnel safety practices (noting that respiratory protection is described in Section 2.I.A.2.b. ii. Standard Working Conditions and Baseline Precautions above).

Not applicable.

- iv. Describe how food is provided to various species (*ad libitum*, limited amounts, types of feeders).

Animals are provided food pellets continuously in cage feeder lids unless other methods are approved in the protocol.

- v. Describe special food quality control procedures including procedures for rotating stock, monitoring milling dates, nutritional quality, bio load, chemical contaminants, etc.

With standard food, the outer plastic wrap and all individual bags are visually checked at the time of delivery. Those with breaks and/or evidence of vermin contamination are not accepted. Food is rotated according to milling dates; outdated food is discarded. Occasionally, special diets are required and stored appropriately and used within mill date.

Teklad and Purina foods are discarded at nine months from mill date. Technical data are available for Purina Animal Chows including analyses of nutritional quality, heavy metals, and pesticides. Autoclavable or irradiated diets are used for animals with compromised immune systems.

b. Drinking Water [Guide, pp. 67-68]

- i. Describe the water source, treatment or purification process, and how it is provided to the animals (e.g., bowls, bottles with sipper tubes, automatic watering, troughs, ponds, streams).

The potable water supply at BNL is obtained from five on-site wells. At the on-site treatment plant, water is treated to remove excessive levels of iron and manganese,

the pH is adjusted to 7.6 – 8.3., and water is filtered through a sand/anthracite mixture and chlorinated at a level not less than 0.3 ppm. The water is routinely tested to ensure that all federal and state drinking water standards are met. The higher pH it is a requirement by Suffolk County Department of Health Services to provide protection against leaching lead and copper out of the pipes. Water is provided to animals either directly from the tap or sterilized (autoclaved) depending upon the study. Rodents receive water continuously in bottles with sipper tubes.

ii. Describe methods of quality control, including monitoring for contaminants.

Potable water at BNL is extensively monitored as required by the Safe Drinking Water Act. The regulatory agency approved monitoring program includes: (1) once every three months, water samples from the distribution system are tested for bacteria and organic compounds directly from sinks in the wash area and staff break room; (2) twice a year a sample from the water treatment plant is analyzed for inorganic solutes and other water quality parameters; (3) every three months, a sample is taken from the water supply in the animal facility and tested for residual chlorine, e-coli, and total coliform bacteria.

iii. If automatic water delivery systems are used, describe how they are maintained and sanitized.

None

c. Bedding and Nesting Materials [*Guide*, pp. 68-69]

i. Describe type(s) and how used for various species.

Ground 1/8" corncob bedding (Anderson Products, Maumee, OH) is used directly for rodents.

ii. Describe bulk bedding storage facilities, if applicable, including vermin control measures.

Bedding, upon entry into the animal facility, is inspected and stored on plastic pallets in a room that has direct access to the clean utility area. Vermin control is an ongoing program of the Grounds Maintenance Division. The room is solid block concrete construction with impervious ceiling, epoxy painted concrete floor and doors fitted with door sweeps to ensure that it remains vermin free.

iii. Describe quality control procedures, including monitoring for contaminants.

The outer plastic wrap and individual bags are visually inspected upon delivery. Bags with breaks and/or evidence of vermin contamination are not accepted. Technical data for corncob bedding, including laboratory analysis for heavy metals and pesticides, are available from the company.

d. Miscellaneous Animal Care and Use Equipment

- i. Describe motorized vehicles and other equipment (e.g., trailers) used for transporting animals, noting the type and how the cargo compartment is environmentally controlled, if applicable.

Heated/air-conditioned vans are available for transporting animals. Depending on the outside temperature, the vehicle's heating or AC controls are adjusted to a comfortable level ten minutes prior to animal loading and transport. All animal cages are covered with micro-isolator lids to reduce allergen exposure to the driver.

- ii. Describe other animal care related equipment used in the animal care program (specialized equipment for exercise or enrichment, high pressure sprayers, vacuum cleaners, tractors, trailers, spreaders, etc.).

Other animal equipment includes: (2) pressure washers; floor scrubber with wet vacuum and floor squeegee; vacuum equipped with HEPA filter for fine particles and dust; wet vacuum with floor drain used to clean tunnel washer tank and various pallet jacks.

e. Sanitation [*Guide*, pp. 69-73]

i. Bedding/Substrate Change

- 1) Describe frequency of contact and non-contact bedding change for each species and enclosure type (solid-bottom or suspended) or pen.

Solid-bottom rodent cages are changed once or twice a week depending upon the number of animals housed per cage.

- 2) Describe any IACUC/OB approved [exceptions](#) to frequencies recommended in the *Guide* or applicable regulations and the criteria used to justify those exceptions.

The only exception is to reduce bedding changes from twice weekly to once a week for mice that fight.

- 3) Note the location where soiled bedding is removed from the cages/enclosures and where clean bedding is placed into the cages/enclosures.

Soiled bedding is removed from cages in Room 11-118, and clean bedding is added to clean cages in Room 11-205. Upon arrival, bedding is moved to the clean utility area (11-205A) and stored on pallets. As needed, individual bags are transferred on a clean cart to the bedding dispenser located in the adjoining room (11-205). Bedding is emptied into the bedding dispenser hopper, and once filled, bedding is dispensed into clean cages directly from the tunnel washer. Autoclaved bedding is provided for immune compromised animals only.

ii. **Cleaning and Disinfection of the Micro- and Macro-Environments**

Note: A description of the washing/sanitizing frequency, methods, and equipment used should be included in **Appendix 14** (Cleaning and Disinfection of the Micro- and Macro-Environment) and **Appendix 15** (Facilities and Equipment for Sanitizing Materials).

- 1) Describe any IACUC/OB approved [exceptions](#) to the *Guide* (or applicable regulations) recommended sanitation intervals.

None.

2) **Assessing the Effectiveness of Sanitation and Mechanical Washer Function**

- a) Describe how the effectiveness of sanitation procedures is monitored (e.g., water temperature monitoring, microbiological monitoring, visual inspections).

Heat sanitation: Water temperature of the rinse cycle on the tunnel washer is set at 180°F and monitored visually by digital readout. A thermocouple shuts off the conveyor belt on the tunnel washer when rinse water is below 180°. Detergent concentration and pH of wash water of mechanical washers are monitored 2 to 4 times per year by Quip personnel. Washing efficiency is monitored weekly using "temp/tape" strips. Strips change color if exposed to water at 180°F.

Chemical sanitation: An aqueous solution of chlorine dioxide (Quiptrol 3000) is automatically dispensed into the tunnel washer during the cage cleaning process.

The AccuPoint ATP Sanitation Monitoring System is used to detect any biological activity after chemical or heat sanitation of caging and equipment is complete. A bacterial monitoring system (Attest™ – 3M) is used for all autoclaves and is monitored for each load for adequate sterilization.

Autoclave tape is added to each load as a visual aid to confirm sterilization temperature. All monitoring records are available.

At a minimum, ATP testing is done at the beginning of the wash load and then again at the end. If bacterial monitoring tests fail, the washer is checked and the cages rewashed.

b) Describe preventive maintenance programs for mechanical washers.

Maintenance is performed under contract from the manufacturer. They are available for repairs at any time.

f. Conventional Waste Disposal [*Guide*, pp. 73-74]

Describe the handling, storage, method and frequency of disposal, and final disposal location for each of the following:

i. Soiled bedding and refuse.

Soiled bedding is removed from cages in the waste dump area. Waste bedding is dumped into a Nu-Aire Animal Bedding Disposal Cabinet/Hood where it is carried along a mechanized screw augur/conveyor to an outside waste container for composting. Other trash is placed in dumpsters that are emptied weekly by Grounds Maintenance Personnel. Waste bedding is composted in a designated area on BNL site.

ii. Animal carcasses.

Carcasses are bagged and placed in a freezer until removed for final disposal by an outside vendor.

g. Pest Control [*Guide*, p. 74]

i. Describe the program for monitoring and controlling pests (insects, rodents, predators, etc.). Include a description of:

- monitoring devices and the frequency with which devices are checked
- control agent(s) used and where applied, and
- who oversees the program, monitors devices, and/or applies the agent(s).

The AV/BLAF Manager, in consultation with investigators, ESH Rep and the Production Services Division, coordinate the pest control program. If an infestation occurs, a qualified pesticide technician working under the direction of a NY State licensed Pesticide Applicator within the Production Services Division, or a qualified pesticide technician working under the direction of a licensed applicator inspects the area. If insecticides are applied, treated areas and

chemicals used are documented and filed with the Facilities & Operations Directorate. Copies are made available upon request. Summary of program for controlling pests:

(1) Areas are inspected to determine the general locality of cockroach/insect infestation.

(2) Live Catch tip traps are used to trap wild mice. The traps are placed near all facility entry doors, inspected daily by BLAF staff, and changed as needed. Findings are reported on the pest control log whenever a trap is changed.

(3) All doors have door sweeps.

Other pest control measures performed by the BLAF personnel:

(1) Open drains are routinely cleaned and sprinkled with bleaching cleanser by Animal Facility Personnel.

(2) Wild and/or escaped rodents are trapped and euthanized.

- ii. Describe the use of natural predators (e.g., barn cats) or guard animals (e.g., dogs, donkeys) used for pest and predator control, if applicable.

None.

- iii. Note how animal users are informed of pesticide use and how animal users may opt out of such use in specific areas.

If insecticides are to be used in animal rooms, it is only after investigators have been fully informed verbally and, in all instances, they clearly have the right of refusal.

h. Weekend and Holiday Animal Care [*Guide*, pp. 74-75]

- i. Describe procedures for providing weekend and holiday care. Indicate who (regular animal care staff, students, part-time staff, etc.) provides and oversees care and what procedures are performed.

Care on weekends and holidays is provided by regular BLAF personnel. Routine responsibilities are to observe all animals for health and assure they have adequate food and water. The facility is always locked and is routinely monitored by Security walk-throughs. .

- ii. Indicate qualifications of weekend/holiday staff if not regular staff.

Regular staff is used.

- iii. Describe procedures for contacting responsible animal care and/or veterinary personnel in case of an emergency.

Telephone numbers of all appropriate contact personnel including veterinarians, technicians, and principal investigators are on-file with the Laboratory Protection Division. Contact information is also included on the instruction sheet listed on individual animal room entry doors. The BLAF Manager or designee is on call 24/7 for facility related issues. The AV is on call 24/7 via cell phone or long range pager for veterinary emergencies.

2. Population Management [Guide, pp. 75-77]

a. Identification

Describe animal identification methods for each species (e.g., microchips, cage/tank cards, collars, leg bands, tattoo, ear tags, brands).

Rodents are identified using colored cage cards that contain the following information: Protocol Number(s), Strain, Species, Born Date, Sex, Date Received, Vendor, Investigator, Telephone Number, Animal(s) ID Number, Experiment and/or Special Instructions.

In certain studies rodents may be individually identified by tail tattoo, ear punch, metal clips or microchips.

b. Breeding, Genetics, and Nomenclature

- i. Describe the program for advising investigators on the selection of animals based on genetic characteristics.

The AV helps investigators obtain the correct strain and/or substrain designation.

- ii. Describe the program for advising investigators on using standardized nomenclature to ensure proper reporting of the identification of the research animals with regard to both the strain and substrain or the genetic background of all animals used in a study.

Ensuring that correct genetic nomenclature is used is an ongoing process. When any new standardized nomenclature rules are put into place, the AV discusses them with appropriate investigators and is included in the protocol review which is then used to procure animals. Currently there are no BNL employees performing rodent studies. External users provide strain designations in their IACUC protocol that have been determined at their home facility.

- iii. Describe genetic management techniques used to assess and maintain genetic variability and authenticity of breeding colonies, including recordkeeping practices (Guide, pp. 75-76).

Not applicable.

- iv. For newly generated genotypes, describe how animals are monitored to detect phenotypes that may negatively impact health and well-being. Note that the methods used to report unexpected phenotypes to the IACUC/OB should be described in section 2.1.B.1.c.ii, “Unexpected Outcomes that Affect Animal Well-Being.”

If genetically modified animals are to be used on a protocol, any known or suspected negative impacts are listed in the protocol, along with any special considerations in animal care that will be required and provided. For novel strains where potential negative impacts are unknown, the animals are closely observed by both research and animal care staff and any abnormalities identified are discussed with the AV and included in the IACUC protocol. There are no breeding programs currently in place at BNL.

III. **Veterinary Care** [Guide, pp. 105-132]

Note: Complete each section, including, where applicable, procedures performed in farm settings, field studies, aquatic environments, etc.

A. **Animal Procurement and Transportation** [Guide, pp. 106-109; Ag Guide, pp. 8; 45; 50-57]

1. **Animal Procurement**

Describe the method for evaluating the quality of animals supplied to the institution (from commercial vendors, other institutions, etc.).

Animal sources are selected on the basis of animal health surveillance reports, method/mode of delivery, and availability of the specific species or strain of animal required. Animals are procured from reputable commercial breeders who extensively monitor for the presence of pathogens. If animals from a non-approved vendor are requested then specific quarantine procedures, diagnostic testing, and/or re-derivation may be required before animals are allowed in the facility. Re-derivation would be done by a contract facility. The AV is consulted if there are any questions regarding the health or quarantine procedures.

2. **Transportation of Animals**

Describe how animals are transported between outside sources and the institution and within the institution, including loading, unloading, level of biosecurity, immune status and specific pathogen status (consider all species, including aquatic and semi-aquatic species).

Purpose-bred animals are transported directly from vendors in environmentally controlled company vehicles. These vendors provide individual/colony animal health and pathogen

information which is routinely reviewed and assessed by veterinary personnel. Animals from collaborating institutions are shipped via airfreight then transported to the facility by approved couriers only after receiving health approval by BNL's AV. Rodents may be transported in personal, climate-controlled vehicles under certain circumstances (vehicle conflict, special request by PI) with prior approval from the facility manager and with specific SOP training. On site, rodents are transported in a specifically designated temperature-controlled van. Prior to intra-institutional transport, rodent cages are changed and covered with micro-isolator lids. The cages are transported on racks through the animal facility dirty corridor to the BLAF loading dock. The animal cage racks are moved out the door approximately six feet to the vehicle, which is either heated or cooled, prior to animal transfer. The animals are individually placed in the van's cargo area which is covered with disposable plastic lined pads. Animals are handled and transported on-site by veterinary staff expertly trained in techniques to prevent zoonotic transmission, cross contamination, and allergen exposure. The appropriate PPE is worn when handling and transporting rodents. No infectious materials or animals have been transported, and no disease outbreaks have occurred. Turtles are transported by personal and or lab vehicle in clean box or plastic container for marking then released back to point of capture. They are also transported to central location for addition/change of radio transmitters when necessary. Box containers are used once and discarded. Plastic containers are washed and air dried between uses. Proper PPE is used when handling and hand washing is required immediately after handling turtles.

B. Preventive Medicine

1. Animal Biosecurity [*Guide*, pp. 109-110]

- a. Describe methods used to monitor for known or unknown infectious agents. Note that if sentinel animals are used, specific information regarding that program is to be provided below.

Individually Ventilated Cage Systems (IVC's) equipped with media filter cards monitor infectious agents through Exhaust Air Dust (EAD) PCR testing on the rack filter or through plenum swab samples. This type of monitoring known as "sentinel-free" is an alternative to traditional methods that test sentinel animals through PCR, serology or whole animal screening. A small number of sentinel rodents are maintained within the IVC rack to agitate the cage dust necessary for testing. Samples are sent to Charles River diagnostic laboratory for viral, bacterial and parasite pathogen testing, 2 times per year, after animal runs are completed. The potential pathogens that are not excluded from the facility are MNV (Mouse Norovirus) and Helicobacter species.

- b. Describe methods used to control, contain, or eliminate infectious agents.

Known health status of animals before receipt; separating clean and dirty side operations and personnel; using disposable gloves and lab coats; proper sanitation

controls; quarantining and separation of newly received animals; ongoing vermin control program and ongoing health monitoring program for rodents.

2. Quarantine and Stabilization [*Guide*, pp. 110-111]

- a. Describe the initial animal evaluation procedures for each species.

Animal cartons received are inspected by BLAF personnel for damage and outer surfaces disinfected before opening. The species, strain, sex, and number of animals are verified, and the animals are visually inspected for any obvious health problems. Rodents from non-commercial vendors are tape tested for parasite ova and treated prophylactically for fur mites. Rodents placed in clean/sanitized cages and housed in clean/sanitized, vacant animal rooms.

- b. Describe quarantine facilities and procedures for each species. For each species, indicate whether these practices are used for purpose-bred animals, random-source animals, or both.

There are no quarantine facilities and all animals are purpose bred.

- c. Describe the required/recommended stabilization period for each species.

An acclimation period of no less than 24 hours is suggested for rodents. Historically this has not been a problem and the acclimation is generally several days.

3. Separation by Health Status and Species [*Guide*, pp. 111-112]

- a. Describe the program for the separation of animals by species, source, and health status. If the animals in different status are not maintained separately, describe circumstances in which mixing occurs and explain the rationale for mixing.

Animals of different species are housed separately.

- b. Describe situations where multiple species may be housed in the same room, area, or enclosure.

Animals from different sources are housed separately except in certain instances where, according to the principal investigator, experimental protocol justifies combining same species animals from different sources. Mice are housed either in IVC's or conventionally in cages with micro-isolator lids.

- c. Describe isolation procedures and related facilities for animals.

Ill rodents are isolated in a separate room depending upon symptoms, housed in a separate cage within the room and treated or euthanized. In cases where infectious disease is suspected, however, some animals may be euthanized for diagnostic purposes and the entire room isolated and treated as indicated.

C. Clinical Care and Management [*Guide*, pp. 112-115]

1. Surveillance, Diagnosis, Treatment and Control of Disease [*Guide*, pp. 112-113]

- a. Describe the procedure(s) for daily observation of animals for illness or abnormal behavior, including:
- the observers' training for this responsibility
 - method(s) for reporting observations (written or verbal)
 - method(s) for ensuring that reported cases are appropriately managed in a timely manner.

Daily observation of animals is done primarily by BLAF personnel and project technicians; as needed less frequent observations are made by the AV. Extensive on the job training, formal training, and verbal instructions from the BLAF Manager, AV and research investigators qualify these employees.

Observations describing the illness or injury are recorded in a sick log book and an identification card labeled "sick", is attached to the cage. All abnormal health observations are reported verbally or by e-mail to the BLAF Manager, the Principal Investigator, or the AV.

- b. Describe methods of communication between the animal care staff and veterinary staff and the researcher(s) regarding ill animals.

Communication between investigators and the animal care staff/veterinarian is accomplished verbally or by e-mail.

- c. Describe the preventive medicine and health management/monitoring programs (e.g., physical examination, TB testing, vaccination, hoof/nail trimming, teeth cleaning/floating, vendor surveillance, use of sentinel animals) for each species.

Rodents: Health surveillance reports from vendors are routinely reviewed. At present all vendors who supply animals to the Laboratory report pathogen free stock. A rodent health program is ongoing. Representative rodents are Maintained in cages within the IVC rack for dust collection and testing through EAD (exhaust air dust plenum) capture method in conjunction with PCR analysis. Samples are sent to Charles River diagnostic laboratory for testing upon completion of animal runs at least two times per year.

2. Emergency Care [*Guide*, p. 114]

- a. Describe the procedures to ensure that emergency veterinary care is continuously available for animals during and outside of regular work hours, including access to drugs or other therapeutics and equipment.

Telephone numbers of all appropriate contact personnel including veterinarians, technicians, and principal investigators are posted in the main facility and are listed on each animal room. In addition, phone numbers are kept on-file with the Laboratory Protection Division. The AV is on call 24/7 via long range pager or cell phone. A back-up Veterinarian, the Clinical Veterinarian at Stony Brook, is available when the AV is out of town. The AV would bring appropriate therapeutics and/or equipment and drugs are accessible on site.

- b. Describe the authority of the Attending Veterinarian or his/her designee relative to the emergency treatment of animals in the program.

The AV has authority to treat any animal or may grant such authority to the BLAF Manager in his absence.

3. Clinical Record Keeping [Guide, p. 115]

- a. Describe the procedure for maintaining medical records and documenting treatment of ill animals including: clinical laboratory findings, diagnoses, treatments, medical progress records, etc. Identify the species for which individual records are maintained and where such records are kept.

All rodents are observed daily for health and well-being, by the animal care staff. Daily observation is recorded by the BLAF staff on rodent room logs. When animals are put on study, the P.I. or technician responsible monitors them for clinical signs related to the experiment. Sick or injured rodents are reported to the Staff Vet and/or the P.I. for diagnoses and course of treatment. Any abnormal observations i.e., not eating or drinking, injury, weight loss, hair loss, etc. is recorded on the BLAF Rodent Sick Log and on the Veterinary Treatment and Observation Plan. Any course of action is documented on the Treatment/Observation Log until the Attending Veterinarian or BLAF Manager has determined that treatment and/or observation is no longer necessary. A 3-part label/card is used to identify the sick animal. The sick/dead card is attached to the cage or animals cage card depending on if it's single or group housed, the dead card shall be affixed to the carcass bag if the animal dies, and the third card is for veterinarian for room and animal identification.

It is the responsibility of the P.I. or technician to respond to the information provided concerning the health status of the research animal. If there is no action taken on the part of the P.I., appropriate treatment or other measures are initiated without regard to the experiment. The P.I. or research technician collects and

records all data concerning individual animal health and treatment.

- b. Identify individual(s) (titles, not necessarily names) responsible for maintaining such records and identify where the records are maintained and who, including the IACUC/OB has access to the records.

Animal records are maintained by the scientific investigators and technicians at their home institutions. IACUC policy ensures that investigator records may be examined by the IACUC, AV or outside inspectors at any time.

- c. Describe the role of the Attending Veterinarian in recordkeeping.

Any procedures, manipulations or treatments performed on an animal are recorded in the animal record by the AV.

4. Diagnostic Resources. Describe available diagnostic methods used in the program including:

- a. In-house diagnostic laboratory capabilities.

Microscope slides, tape test for rodent pinworm ova.

- b. Commercially provided diagnostic laboratory services.

Commercial diagnostic services are used to monitor the health status of rodent colonies and other groups of animals as requested by investigators or the AV or required by experimental protocol. These services are utilized as needed. They include:

- i. Gross and microscopic examination
- ii. Microbiology, including fecal cultures
- iii. Parasitology (internal and external)
- iv. Serology for viral, and mycoplasma pathogens
- v. CBC and blood chemistry.

- c. Necropsy facilities and histopathology capabilities.

Necropsy facilities are available. The vast majority of animal deaths are the result of euthanasia approved by protocols. Histopathology for unexpected death is done commercially.

- d. Radiology and other imaging capabilities.

Not applicable.

5. Drug Storage and Control

- a. Describe the purchase and storage of controlled and non-controlled drugs.

All controlled substances are purchased, stored and dispensed through Brookhaven Science Associate's (BNL's) license with the Drug Enforcement Agency (DEA) to operate a dispensary. Only compounds in Schedules II-V are utilized. One individual, specified through a Power of Attorney from BSA, is authorized to order controlled substances and to dispense them to particular researchers. DEA background checks and controlled substance training are required for access to and handling of controlled substances. More detailed institutional guidelines and requirements are outlined in Brookhaven's online Standards Based Management System (SBMS) (Using Controlled Substances in Research Subject Area).

- b. Describe record keeping procedures for controlled substances.

Specific institutional guidelines and requirements for record keeping are outlined in Brookhaven's online Standards Based Management System (SBMS) procedures (Using Controlled Substances in Research Subject Area). An authorized agent of BSA is on staff to order, dispense and manage controlled substances.

D. Surgery [Guide, pp. 115-123]

1. Pre-Surgical Planning [Guide, p. 116]

Describe the process(es) used to ensure adequate pre-surgical planning, including: identifying personnel; locating equipment, supplies, veterinary involvement for selecting analgesic and anesthetic agents and facilities; planning; and pre- and post-operative care.

The AV and the IACUC must approve all surgical procedures prior to the start of work. All Principal Investigators and research technicians who perform surgery are trained in pre-operative and post-operative care.

2. Surgical Facilities [Guide, pp. 116-117, 144-145]

List building name(s) and room number(s) or other locations (coded, if confidential) where surgical procedures are performed. For each, describe:

- the type of species (including rodents, fish, agricultural species, etc.)
- nature of procedure(s) (major/minor/emergency, survival and non-survival, etc.)
- the amount of use [heavy (daily), moderate (weekly), or light]
- major surgical support equipment available (gas anesthesia machines, respirators, surgical lights, etc.)
- facilities for aseptic surgery, surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery

- construction features of the operating room(s), including interior surfaces, ventilation, lighting, and fixed equipment used to support surgical procedures and other means of enhancing contamination control

Note: If preferred, the information requested in this section may be provided in Table.

Minor rodent surgeries are performed aseptically in a designated IACUC approved animal procedure room or research lab. These spaces are inspected by the IACUC semiannually. Use is very light. . There are no operating rooms or major surgical equipment.

3. **Surgical Procedures** [*Guide*, pp. 117-118]

- a. Describe the criteria used to differentiate major from minor survival surgery, including classification for certain procedures (e.g., laparoscopic technique).

A major surgical procedure is one in which a body cavity is penetrated and opened or when physiological function is impaired. A minor surgical procedure is one in which the body cavity is not exposed and physical function is not impaired.
All surgery is performed following the Surgery SOP.

- b. How is non-survival surgery defined?

A non-survival surgical procedure is a procedure where an animal is euthanized while still under anesthesia.

4. **Aseptic Technique** [*Guide*, pp. 118-119]

- a. Describe procedures, equipment, and protective clothing used for aseptic surgery. Include patient and surgeon preparation.

All surgical instruments are sterilized prior to use on any species. In addition, disposable gowns, sterile gloves, caps and face masks are worn when performing procedures. The surgical area is clipped and surgically scrubbed with a disinfecting agent (betadine/70% alcohol) and a sterile drape is then used to cover the rest of the animal.

- b. Describe methods used to sterilize instruments and protective clothing, including a description of approved [liquid sterilants](#) and instrument exposure time(s) required for each, if applicable.

Instruments and protective clothing are sterilized in an autoclave at 250°F for fifteen minutes. Effectiveness of sterilization is verified by use of the Attest Biological

monitoring system. All results are recorded in a log book. Serial surgeries are not conducted at BNL. .

- c. Describe methods for instrument re-sterilization between serial surgeries.

N/A

- d. Indicate how effectiveness of sterilization is monitored.

N/A

- e. Describe surgical support functions provided by the program to investigators.

A project technician or Principal Investigator who is qualified by a veterinarian would perform surgical support services for experimental surgery. Animals would be anesthetized and prepped in an IACUC approved room. Aseptic techniques would be employed, i.e., sterile prep of animal and surgical area, sterile surgical attire and sterile surgical instruments would be used. Animals would be observed until recovery. Post-surgical treatment would routinely include the administration of antibiotics (see below) and analgesics.

5. Intraoperative Monitoring [*Guide*, p. 119]

Describe monitoring and recording requirements for each species, including the type of record(s) maintained. Also note monitoring of anesthesia during non-survival procedures.

During surgery all animals are monitored by investigator personnel. The specific surgical monitoring that will be performed varies between procedures and is detailed, and approved, in the animal care protocol. All animals are monitored for anesthetic depth, cardio/respiratory function, and other vital signs such as mucous membrane color and capillary refill time. Animal surgical records are maintained by investigator personnel.

6. Postoperative Care [*Guide*, pp. 119-120]

Describe the postoperative care program, including who is responsible for overseeing and providing the care, types of records maintained (e.g., perioperative), where the records are maintained, etc.

Post-surgical care for animals is the responsibility of the project personnel, in coordination with the BLAF Manager and AV. Post-surgical care would include daily observation, systemic administration and local application of antibiotics and analgesics and/or other drugs as needed. Records for animals are maintained by the researchers. Antibiotics are not routinely used post-surgery except for when asepsis is broken or gastrointestinal surgery is performed. Analgesia is always provided unless scientific justification is provided to withhold its use.

E. Pain and Distress [*Guide*, pp. 120-121]

1. Describe how and by whom pain and distress are assessed.

In the pre-planning stage, procedures that may result in pain and distress are identified, reviewed, and approved by the AV, prior to approval by the IACUC. The protocol form also includes specific clinical parameters that will be monitored during the experimental period. All animals are observed at least daily.

2. Describe training programs for personnel responsible for monitoring animal well-being, including species-specific behavioral manifestations as indicators of pain and distress.

BLAF personnel receive hands-on, web-based and formal training to recognize, evaluate and monitor animal well-being for multiple species. BLAF personnel participated in the online AALAS webinars “Evidence-based Pain Management and the Physiology of Pain” and “Barbering and Ulcerative Dermatitis” to enhance knowledge of potential pain and distress in animals.

F. Anesthesia and Analgesia [*Guide*, pp. 121-123]

1. List the agents used for each species.

Note: If preferred, this information may be provided in Table or additional Appendix.

Mice: Sodium Pentobarbital, Buprenorphine, Ketorolac, Isoflurane
Rats: Ketamine/Xylazine, Sodium Pentobarbital, Buprenorphine, Ketorolac, Isoflurane.
Ketamine/Xylazine is mixed by individual researchers and maintained in a designated lockbox.

2. Describe how the veterinarian provides guidance and advice to researchers concerning choice and use of anesthetics, analgesics or other pain moderating methods.

Advice and guidelines for the use of analgesic and anesthetic agents are provided verbally and/or written to investigators by the AV, or an approved trainer, and through on-line course instruction. This may occur prior to or at the time of IACUC protocol submission.

3. Describe the monitoring of the effectiveness of analgesics, including who does the monitoring. Include in the description any non-pharmacologic means used to diminish pain and distress.

The trained PI, research technician or other staff monitor the effectiveness of analgesics and anesthetics in compliance with parameters outlined in the approved IACUC protocol.

4. Describe how the veterinarian(s) and the IACUC/OB evaluate the proposed use of neuromuscular blocking agent to ensure the well-being of the animal.

The use of neuromuscular blocking agents must be scientifically justified in the IACUC protocol, and the method of monitoring anesthetic depth must be clearly stated as well. There are no protocols currently approved with use of neuromuscular blocking agents.

5. Describe policies and practices for maintaining and ensuring function of equipment used for anesthesia.

Anesthesia vaporizer equipment is calibrated at least every other year; proper function of equipment is checked prior to use; all anesthetic and analgesic agents are used before the expiration date.

G. Euthanasia [*Guide*, pp. 123-124]

1. Describe approved methods of euthanasia, including humane slaughter (for additional guidance, see pertinent [AAALAC Reference Resources](#)). Include:
 - consideration of species, age, condition (e.g., gestational period, or neonatal) and
 - location(s) for the conduct of the procedure.

Note: If preferred, this information may be provided in Table or additional Appendix.

Mice: Carbon dioxide (100% at a 60% air replacement per minute); IP Sodium Pentobarbital or pentobarbital-based euthanasia solution; Anesthesia followed by cervical dislocation; Perfusion. Decapitation is allowed with scientific justification. It is performed by skilled personnel using a guillotine that is serviced at least quarterly.

Rats: Carbon dioxide (100% at a 60% air replacement per minute); IP Sodium Pentobarbital or pentobarbital-based euthanasia solution; Anesthesia followed by exsanguination. Decapitation is allowed with scientific justification. It is performed by skilled personnel using a guillotine that is serviced at least quarterly.

Wildlife: When live capture or live handling of wildlife occurs, euthanasia is rarely necessary due to the nature of the research. However, for small animals like birds, bats, and small mammals that are captured through use of mist nets or live traps, cervical dislocation will be used. Larger wildlife, that may be injured during capture or during investigations, are transported to a local emergency veterinary hospital to assess the need for euthanasia or rehabilitation. If euthanasia is required, it is carried out by veterinary staff at the animal hospital.

Secondary methods used to assure death of an animal are listed in each protocol.

2. Describe policies and practices for maintaining and ensuring function of equipment used for euthanasia.

CO2 tanks are pressure tested and amounts are verified by visual inspection of gauges. Clear delivery lines are inspected and replaced as needed.

3. Describe the methods used to confirm death of an animal.

Personnel trained to perform euthanasia confirm death by the absence of vital functions such as no reflexes or heartbeat and/or cervical dislocation.

IV. Physical Plant [Guide, pp. 133-155]

A. Facilities Overview

Provide a brief introduction to the animal housing and use facilities. Note that this overview should augment the information provided in **Appendix 2** (Summary of Animal Housing and Support Sites), which includes area, average daily census, and person responsible for each site. Please use consistent terminology for the buildings/areas/sites described in the Location section of the Appendix. Please do not repeat information, but supplement the descriptions provided elsewhere to assist the reviewers understanding of the interaction between facilities, special housing locations, and separate procedural areas.

Bldg. 490 (BLAF): Currently, 4 heated and air-conditioned rooms, 11-147 B, 11-147 C, 11-241 and 11-242 (a total of 1070 square feet) are used for housing animals.

Bldg. 958 (NSRL): The Animal Area is located on the north side of Building 958 with 3 heated and air-conditioned holding rooms, totaling 593 square feet.

B. Centralized (Centrally-Managed) Animal Facility(ies)

In this section, describe each centralized or centrally-managed animal housing and use facility. Include in **Appendix 3** the floor plans of each on 8.5" x 11" or A4 paper. Ensure that the drawings are legible and the use of each room is indicated (animal housing, procedure room, clean cage storage, hazardous waste storage, etc.). Note that a separate section for describing "satellite housing areas" is included below.

Separately describe **each** Location or Animal Facility, addressing each of the features outlined below (1-8). A complete description of each must be provided; however, common features among locations or facilities may be indicated as such and do not need to be repeated.

1. General arrangement of the animal facilities (conventional, clean/dirty corridor, etc.).
2. Physical relationship of the animal facilities to the research laboratories where animals may be used.
3. Types of available animal housing spaces used, such as conventional, barrier, isolation/quarantine, hazard containment (infectious, radioactive, chemical), "animal

cubicles” or facilities specifically designed for housing certain species such as ponds, pastures, feedlots, etc.

4. Finishes used throughout the animal facility for floors, walls, ceilings, doors, alleyways, gates, etc. (note any areas that are not easily sanitized and describe how these are maintained).
5. Engineering features (design, layout, special HVAC systems, noting exhaust air treatment, if applicable) used in hazardous agent containment.
6. Security features, such as control of entry, perimeter fences, gates, entryways, cameras, guards; identify and describe exceptions for individual facilities or areas incorporating fewer or additional security features than the general features described.
7. Consideration for facilities with exterior windows, if applicable, including management of environmental conditions (i.e., temperature and photoperiod control) and potential security risks.
8. Storage areas for flammable or hazardous agents and materials (e.g., disinfectants, cage-washing chemicals, pesticides, fuel).

Rodents are housed conventionally and in ventilated cage systems in rooms serviced by a clean/dirty corridor system.

The BLAF is located in the same building and is adjacent to most research laboratories. NSRL is approximately 1 mile from the BLAF.

BLAF (Bldg. 490) Corridors are 6-10 ft. wide with floors composed of epoxy painted concrete. A continuous concrete baseboard covering extends approximately 6 inches up the wall. All rooms have epoxy painted sheetrock ceilings and steel doors.

NSRL (Building 958) The entire Animal area has “Stonhard” flooring. All rooms have epoxy painted sheetrock ceilings and steel doors.

BLAF: Animal rooms are serviced by a “Trane” HVAC system and are supplied with once-through-air at a rate of 10-19 air changes per hour. “Logic” technology is used to continuously monitor room environmental conditions.

NSRL: The Animal Area is serviced by a dedicated HVAC system which supplies a once-through-air rate of ~12 air changes per hour for each of the 3 animal rooms. An automated control system is used to continuously monitor room environmental conditions in each animal room. The HVAC system uses independent electric reheat for each room and common steam humidification controlled/monitored by the automated control system to provide temperature and humidity control. Air flow balance (pressure) in each room is controlled manually with manual control dampers in the HVAC ductwork.

The BLAF is locked and LPD personnel routinely walk through the facility during off hours to ensure all doors are secured.

The NSRL is locked and security cameras feed live to the Laboratory Protection Division who maintains a list of all personnel approved to use the facility. Key card access is required to enter the building.

BLAF: The Animal Area does not have any exterior windows. All rooms have interior windows that are covered with a red film to block evening emergency lighting which maintain appropriate photo periods for rodents.

NSRL: The Animal Area does not have any exterior windows. All interior windows are covered with a red film to block evening emergency lighting which maintain the appropriate photo periods for rodents. Security risks are minimal due to our 24-hour surveillance system. Cage washing chemicals are kept to a minimum and stored in the main facility cage-wash area Room 11-105. Chemical/container: Enviro-Kleen 1200 15 gallon drum; Quiprol 3000/30 gallon drum; Sani-Plex 128 M disinfectant/1 gallon container. A chemical room (9-267) located outside the animal facility in Building 490 is used to store hazardous, non-hazardous, and flammable chemicals. In addition, flammable chemicals are double contained and stored in BNL safety approved flammable cabinets.

C. Satellite Animal Housing Facilities

In addition to the Appendices summarizing Heating, Ventilation, and Air-Conditioning (**Appendix 11**) and Lighting Systems (**Appendix 16**), summarize animal housing areas that are not centrally-managed or maintained in (**Appendix 17**), "Satellite Animal Housing Areas."

1. Describe the criteria used to determine/define a "Satellite Animal Housing Area," which may include remote housing facilities or laboratories temporarily or consistently housing animals.

Criteria would be whether the area is centrally-managed or maintained.

2. Describe the process used by the IACUC/OB to authorize, provide oversight of, and ensure compliance with *Guide* standards for the housing of animals outside of centrally-maintained facilities. Include a description of Attending Veterinarian access and physical security.

Not applicable.

D. Emergency Power and Life Support Systems

Note: Complete a Heating, Ventilation, and Air-Conditioning (HVAC) Summary (**Appendix 11**) and Lighting Summary (**Appendix 16**) for each Location described in the Summary of Animal Housing and Support Sites (**Appendix 2**).

1. Power [*Guide*, p. 141]

For each Location, Centralized Animal Facility, and Satellite Housing Facility, provide a brief description of the following:

- Availability of [emergency power](#) and if so, what electrical services and equipment are maintained in the event the primary power source fails.
- History of power failures, noting frequency, duration, and, if emergency power was not available, steps taken to ensure the comfort and well-being of the animals present and the temperature extremes reached in animal rooms during the failure.

BLAF (Bldg. 490): In the event of primary power failure, emergency power is provided to the entire animal facility. This includes all ventilation, heating, air conditioning systems. NSRL (Bldg. 958): Emergency power is provided to the emergency lighting only. In the event of a prolonged power failure, the animals would be transferred to BLAF. Backup generators are tested monthly. There is no history of power failure.

2. **Other System Malfunctions.** If not previously reported, describe animal losses or health problems resulting from power, HVAC, or other life support system (e.g., individually ventilated cages) failures, and mechanisms for reporting such incidences. [AAALAC International Rules of Accreditation](#) (Section 2.f).

None.

E. Other Facilities [*Guide*, pp. 144, 150]

1. **Other Animal Use Facilities** [*Guide*, pp. 146-150]

Describe other facilities such as imaging, irradiation, and core/shared behavioral laboratories or rooms. Include a description of decontamination and methods for preventing cross-contamination in multi-species facilities.

Not applicable.

2. **Other Animal Program Support Facilities**

Describe other facilities providing animal care and use support, such as feedmills, diagnostic laboratories, abattoirs, etc.

Not applicable.

According to the privacy principles on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, we wish to advise you that the personal data in the Program Description will become part a permanent file owned by AAALAC International, and that can be shared with AAALAC International offices and representatives in order to perform an evaluation of the institution's animal care and use program and provide accreditation services. The institution has the option of exercising rights of data access, rectification, erasure, restriction and opposition at: accredit@aaalac.org

Appendix 1: Glossary of Abbreviations and Acronyms

Please provide a Table defining abbreviations and acronyms used in this Program Description.

Abbreviation/Acronym	Definition
AV	Attending Veterinarian
BLAF	Brookhaven Laboratory Animal Facility
BNL	Brookhaven National Laboratory
BSA	Brookhaven Science Associates, LLC
DOE	Department of Energy
ESH	Environment, Safety and Health
ESR	Experimental Safety Review
IO	Institutional Official
NSRL	NASA Space Radiation Laboratory
OMC	Occupational Medicine Clinic
PI	Principal Investigator
PPE	Personal Protective Equipment
SBMS	Standard Based Management System

Appendix 2: Summary of Animal Housing and Support Sites

Briefly summarize in the following Table the animal facility or facilities, noting the number of areas in which animals are housed (buildings, floors, farms, satellite housing facilities, etc.), the total square footage/metres and/or acreage for animal care and use, and the total square footage/metres and/or acreage for necessary support of the animal care and use program covered by this Description (water treatment plant/area if housing aquatic or amphibian species, cage washing facilities, service corridors, etc. and additional areas to be considered are enumerated in the *Guide*). Detailed information for satellite housing facilities is requested in Appendix 17. Include only one line entry for satellite housing facilities in this table to provide the total square footage for all satellite housing areas listed in Appendix 17. If more than one facility/site, note the approximate distance (yards/miles or meters/kilometers) to each facility from a reference point such as from the largest animal facility. A campus/site map (with a distance scale) may be included as an additional Appendix (Appendix 2.1) to provide this information. See [Instructions, Addendum A - Animal Facility Square Footage/Metres Compilation Form](#) for guidance in calculating the size of your animal care and use program.

Animal Housing and Support Sites						
Location (building, site, farm name, etc. ^a)	Distance from main facility ^b	Animal Housing Approx. ft ² /m ²	Support/ Procedures Approx. ft ² /m ² or acreage	Species Housed	Animal Census by Species Approx. Daily	Person in Charge of Site
Bldg. 490 BLAF	Main	1072	Corridors - 4858 Support rooms - 3687	Mice Rats	1420 100	M. Petry
Bldg. 958 NSRL	1.5 miles	578	Corridors – 249	Mice Rats	As per study	M. Petry
Satellite Housing Facilities Total (Expand in Appendix 17)						

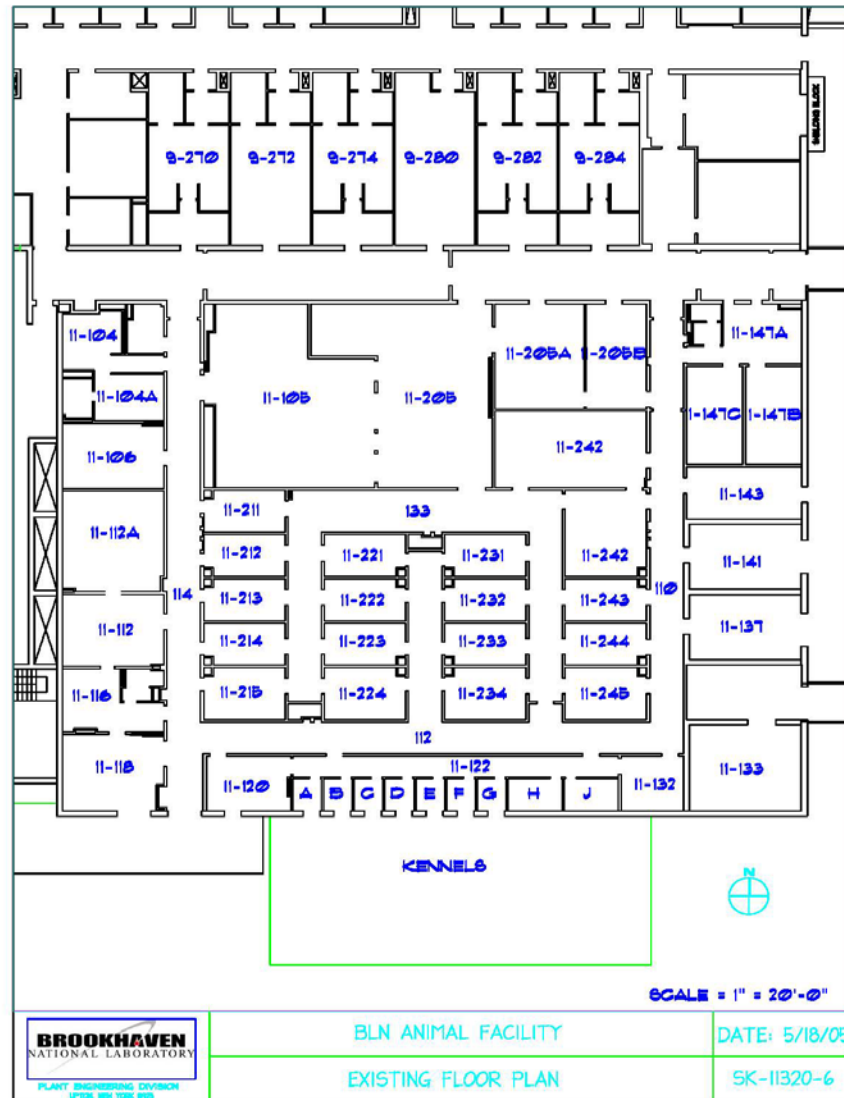
Subtotals (ft²/m²):	1650	8794	
TOTAL Acreage:	10,444 square feet		
TOTAL Animal Housing/Support Procedures (excluding acreage):			
	(please specify ft² or m²)		

^aPlease state name and/or use acronyms described in **Appendix 1** for building names, if not coded for confidentiality.

^bCampus or site map(s) may also be provided in lieu of this information.

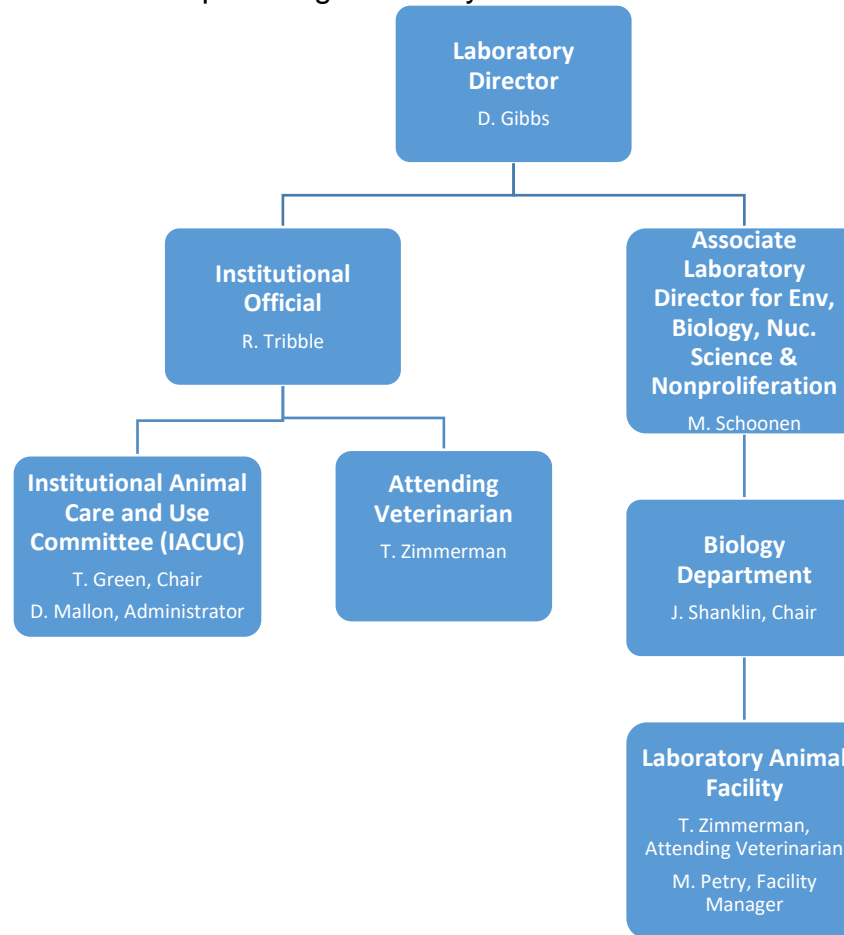
Appendix 3: Line Drawings

Provide floor plans of each centralized animal housing facility. Plans should be provided on 8.5" x 11" or A4 paper. Ensure that the drawings are legible, including room numbers if used, and the use of each room is indicated (animal housing, procedure room, clean cage storage, hazardous waste storage, etc.) either directly on the drawing or in a Key/Table.



Appendix 4: Organizational Chart(s)

Provide an accurate, current, and detailed organization chart or charts that detail the lines of authority from the Institutional Official to the Attending Veterinarian, the IACUC/OB, and personnel providing animal care. If applicable, include personnel responsible for managing satellite housing areas/locations and depict the reporting relationship between the Attending Veterinarian and other(s) having a direct role in providing veterinary care.



Appendix 5: Animal Usage

In order to assist the site visitors in their evaluation of the animal care and use program, please provide the information requested below. Information should be provided for all animals approved for use in research, teaching or testing, including those which may be used or housed in laboratories outside the animal care facility. Of particular interest is information on those animals which are used in research projects involving recovery surgical procedures, behavioral or other testing requiring chairing or other forms of restraint, or exposure to potentially hazardous materials. An alternate format is acceptable as long as the information requested is provided.

Project/Protocol Title	IACU C/OB Number	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Category (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
The Effect of Exposure to Heavy Particles	150	Rabin	Rats	1170	B						
Charged Particle Radiation-Induced Effects in Mice	230	Chang	Mice	704	A						
Neurogenesis and Cognition in Human apoE Transgenic Mice	329	Raber	Mice	816	B						
An Animal Model for Assessing Space Radiation Risks	337	Davis	Rats	600	B			X			
Identification of the Changes in the Brain Proteome Associated with HZE-Induced Neurocognitive Impairment	339	Britten	Rats	900	B						
Mouse Models Approach for Intestinal Tumorigenesis Estimates	345	Fornace	Mice	6180	A						
Animal Holding Protocol	349	Zimmerman	Mice/Rats		A						

Appendix 5: Animal Usage

Project/Protocol Title	IACU C/OB Number	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Category (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Fish and Wildlife Surveys	371	Green	Small mammals Birds Turtles Fish Deer Amphibians Reptiles Meso-carnivores	1000 9000 300 3300 3600 600 500 200	A/B						
Mechanistic Analysis of Particle Radiation-Induced Carcinogenesis Using Validated Mouse Glioma Models	404	Burma	Mice	2100	B						
HZE Particle Radiation and Lung and Colon Cancer Pathogenesis	417	Shay	Mice	490	A						
Effects of Heavy Ions on Neural Precursor Cells	447	Limoli	Mice	2924	B						
Molecular and Cellular Mechanisms of Space Radiation-Induced Changes in Hippocampal Dependent Behavior	453	Eisch	Mice	2700	B						
Biodosimetry and Cancer Risk Prediction to Space Radiation	472	Jacob	Mice	600	B						

Appendix 5: Animal Usage

Project/Protocol Title	IACU C/OB Number	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Category (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
The Role of Oxidative Stress and Inflammation on Synaptic Functions After Exposure to space Radiation	478	Rosi	Mice	264	A						
Determination of Risk for and Occurrence of Heart Disease from Space Radiation	482	Baker	Rats	430	B						
Do Zebrafish Exhibit Cataract Formation from Irradiation to Low-LET or Galactic Cosmic Radiation?	497	Higginbotham	Zebrafish	6840	A					X	
Facility Rodent Health Screening	499	Zimmerman	Mice Rats	15 15	A						
WFIRM Translational Research Institute for Space Health (TRISH) Program	500	Porada	Mice	672	B						
Space Relevant Radiation Induced Cardiovascular Disease Risk Thresholds: Effect of Gender on the Outcome	502	Goukassian	Mice	1620	B						
Sex- and ApoE-Specific Late CNS and Cardiovascular Effects on Space Radiation	504	Lemere	Mice	468	A						

Appendix 5: Animal Usage

Project/Protocol Title	IACU C/OB Number	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Category (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Evaluation of CG4419 as a Pharmaceutical Countermeasure for Space Radiation Induced Lung Carcinogenesis	505	Story	Mice	4738	A/B						
miRNA Signature Detection and Countermeasures Against HZE Radiation Exposure for Tissue Degeneration	506	Beheshti	Mice	300	B/C				X		
γ -Tocotrienol as a Countermeasure against High-Energy Charged Particle-Induced Carcinogenesis	507	Boerma	Mice	578	B						
Gene Therapy Countermeasures for Detrimental Effects of Space Radiation (Rat Neonatal Cardiomyocytes)	508	Bowles	Rats Rat Pups	10 100	A						
Gene Therapy Countermeasures for Detrimental Effects of Space Radiation (Mice)	509	Bowles	Mice	240	A						
Countermeasures Against Adverse Effects of Space	510	Rithidech	Mice	2272	B						
Brain Photomodulation after Whole Body Irradiation	511	Allen	Mice	480	B						

Appendix 5: Animal Usage

Project/Protocol Title	IACU C/OB Number	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Category (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Protection from Space Radiation-Induced Carcinogenesis and CNS Damage with Melanin-Containing Food and Materials	512	Dadachova	Mice	1480	B						
Determine the Relative Biological Effectiveness (RBE) for 12C Ions Compared to X-Rays	513	Story	Mice	226	B						
Responses of the Nervous System to Chronic, Low Dose Simulated Cosmic Rays	514	Nelson	Mice	708	B				X		
Space Radiation-Induced Persistent Estrogenic Response and Risk of Breast Cancer Development	515	Fornace	Mice	936	A						
Oxidative Stress and the Neuroconsequences of Spaceflight Radiation – Immune Dysregulation and Antioxidant Dietary Countermeasures Efficacy in Mice	516	Ronca	Mice	450	B/C				X		
Probing the Synergistic Effects of Radiation, Altered Gravity and Stress	517	S. Rosi	Mice	384	A/C				X		

Appendix 5: Animal Usage

Project/Protocol Title	IACU C/OB Num ber	Principal Investigator	Species	Total Number of Animals Approved	Pain & Distress Categor y (1)	Special Considerations (use checkmark if applicable)					
						SS (2)	MSS (3)	FFR (4)	PR (5)	HA U (6)	NCA (7)
on Behavioral, Cognitive and Sensorimotor Functions to Predict Performance Decrement in Astronauts											

(1) If applicable, please provide a description / definition of any pain/distress classification used within this Appendix in the space below. If pain/distress categories are not used, leave blank.

(2) Survival Surgery (SS)

(3) Multiple Survival Surgery (MSS)

(4) Food or Fluid Regulation (FFR)

(5) Prolonged Restraint (PR)

(6) Hazardous Agent Use (HAU)

(7) Non-Centralized Housing and/or Procedural Areas (NCA), i.e., use of live animals in any facility, room, or area that is not directly maintained or managed by the animal resources program, such as investigator laboratories, department-managed areas, teaching laboratories, etc.

Pain/Distress Classification Description/Definition, if applicable:

LEVEL A: No Pain or Distress: Animals will be euthanized without any treatments or manipulations; OR animals will undergo irradiation with unrestricted movement and no anesthesia and without anticipated subsequent effects while at BNL.

Level B: Relieved or momentary pain or distress: Momentary pain or potential pain or distress relieved by pharmacologic, behavioral or other means, e.g., injection of any substance including anesthetics, post- procedural analgesics, behavioral conditioning, restraint or minor pain/distress and medical treatment of disease states.

Level C: Unrelieved or sustained pain or distress: Any procedure that would cause more than momentary or slight pain or distress, e.g., chronic untreated disease states, pain research.

Appendix 5: Animal Usage

In the Table below, provide an approximate annual usage for all species:

Animal Type or Species	Approximate Annual Use
Mice	8800

Animal Type or Species	Approximate Annual Use
Rats	700

[Create additional rows by pressing TAB in the bottom-right box.]

Appendix 6: Personnel Medical Evaluation Form

BROOKHAVEN NATIONAL LABORATORY OCCUPATIONAL MEDICINE CLINIC

BASIC ANIMAL RESEARCHER PROTOCOL

NAME:

CHART #:

Protocol created 5/02, Rev 5/04, Rev 11/04, 3/17

Synonyms: BNL employees working with non-primate animals
Actions for this Protocol:
Protocol to be done at time of the annual physical Tetanus vaccination per CDC guidelines
Animal Handler History Form Physical Exam
Routine Lab Work
Animal Fact Sheet distributed to each employee

Tetanus and Diphtheria (Td) booster: Booster every ten years unless contraindicated. Contraindications include first trimester of pregnancy, history of local or neurological reaction to previous dose.

Appendix 6: Personnel Medical Evaluation Form

BROOKHAVEN NATIONAL LABORATORY OCCUPATIONAL MEDICINE CLINIC

Animal Handler History Form

EMPLOYEE COMPLETE

NAME: CHART #:

1. Animal species contacted at work (Check all that apply):

Dogs	Cats	Rabbits	Sheep	Pigs	Mice	Rats
Hamsters	Guinea Pigs	Amphibians	Fish	Other		

2. Estimated Animal Contact (hrs/week):

3. Do you use any biological agents (e.g., bacteria, viruses, recombinant DNA, mosquitos, ticks) in your animal work?

No Yes, list agents to be used

4. Do you use any radioisotopes in your animal work?

No Yes Isotope(s) and approximate activity (if known)

5. Do you have frequent contact with animals outside work?

No Yes, list

6. Does exposure to animals cause you any asthma or allergic symptoms?

No Yes, explain

7. Are you immunosuppressed (problem with immune system)?

No Yes, specify: splenectomy, taking steroids, chemotherapy, blood disorder, immune disorder, other

8. Do you have any back pain, back problems or difficulty lifting?

No Yes, explain

9. Date of last tetanus shot:

Employee Signature Date

OMC Physician Signature Date

Appendix 6: Personnel Medical Evaluation Form

BROOKHAVEN NATIONAL LABORATORY OCCUPATIONAL MEDICINE CLINIC

BASIC ANIMAL RESEARCHER PROTOCOL

NAME: CHART #:

Protocol created 5/02, Rev 5/04, Rev 11/04

Synonyms: BNL employees working with non-primate animals. Actions for this Protocol:
Protocol to be done at time of the Recheck Exam Tetanus vaccination per CDC guidelines
Animal Handler History Form Physical Exam
Routine Lab Work
Animal Fact Sheet distributed to each employee

Animal Handler History Form reviewed

Animal Fact Sheet reviewed with employee

Tetanus and Diphtheria (Td) booster: Booster every ten years unless contraindicated. Contraindications include first trimester of pregnancy, history of local or neurological reaction to previous dose.

Date of last Td booster:

Qualified/Completed

Not qualified (Review with OMC Manager)

Pending/Other (Review with OMC Manager)

OMC Physician Signature

Date

Appendix 6: Personnel Medical Evaluation Form

BROOKHAVEN NATIONAL LABORATORY Occupational Medicine Clinic

Infectious Disease Risk and Prevention for Animal Handlers

This information is prepared to increase the understanding of BNL staff who work with animals. It is provided by the Occupational Medicine Clinic (OMC) at Brookhaven National Laboratory. You may contact the OMC on extension 3670 if you have any questions about the information here, or other concerns about the risk of infectious disease in animal handlers.

A number of diseases can be transmitted between animals and humans. However, the current prevalence of such diseases in animals in a modern research animal facility is quite small. This is because of the care taken to make sure animals are free of disease by screening out sick animals, vaccinating animals against vaccine-preventable diseases, preventing exposure of healthy laboratory animals to wild or sick animals, and generally maintaining healthy breeding colonies of laboratory animals.

However, the above measures cannot guarantee that all laboratory animals will be free of diseases that can be transmitted to humans. Therefore, laboratory workers must take additional measures to protect themselves.

This information describes diseases that can be transmitted from laboratory animals of the type in use at BNL to humans, and preventive measures that can help reduce risk to personnel. Information regarding appropriate vaccinations for animal handlers, including those which prevent transmission of infectious disease to animals, is also covered.

Tetanus:

Any type of deep puncture wound from any animal can put an individual at risk of tetanus, which is a serious and frequently fatal disease. Thus, animal bites or deep scratches can put an animal worker at risk of tetanus.

Preventive measures:

1) Tetanus vaccination: Receiving regular tetanus vaccination boosters according to CDC guidelines can greatly reduce the risk of tetanus. 2) Prompt wound care can also reduce risk of infection with tetanus and other infectious agents.

Diseases that can be transmitted from rodents (mice, rats)

Hantavirus:

A serious viral infection that can cause fever, muscle aches, gastrointestinal complaints respiratory distress and fatal lung disease. It is transmitted through airborne mouse feces and urine.

Preventive measures:

BNL screens its mouse colonies to make sure that mice are free of Hantavirus. General good housekeeping and disinfecting surfaces contaminated with mouse urine and feces are also helpful.

Rat-bite fever:

A flu-like illness which may occur 3-10 days after a rat bite, and marked by fever, joint and muscle pain, followed by a rash, mainly on the hands and arms. This disease is rare in the U.S. but has been reported after the bite of a laboratory rat.

Preventive measures:

Safe work practices and use of personal protective equipment to prevent rat bites can prevent the illness. The disease responds to antibiotics, and prophylactic antibiotics may be indicated after a particularly high-risk rat bite. Antibiotics can also be used to treat the illness if it occurs.

Appendix 6: Personnel Medical Evaluation Form

Laboratory Animal Allergy (LAA) is a significant occupational health concern for many animal care workers, staff, scientists, and technicians engaged in the care and use of laboratory animals. LAA is a hypersensitivity reaction upon exposure to a laboratory animal, its fur or dander, urine, saliva or other body tissues. Symptoms range from mild (with little effect on the ability to work) to severely debilitating (preventing further work near the causative allergens). LAA often includes upper respiratory symptoms like sneezing and itchy or running nose and eyes. Lower respiratory symptoms may include wheezing, shortness of breath, and asthma or a feeling of chest tightness. Skin symptoms may include the appearance of red, raised, itchy wheals after contact with animals, their tissues, urine, saliva, skin flakes (dander), or even dusts contaminated with their waste products.

A survey of recent articles suggest that 13-44% of staff working with laboratory rodents and rabbits will report incidences of allergies to laboratory animals. Animal allergy is common in workers exposed to small animals such as mice, rats, gerbils and guinea pigs. These allergies may be caused by dander and debris from the skin and fur of an animal, but more recent studies suggest that exposure to animal urine, saliva and fecal matter may be equally or more important. Studies in mice suggest that prealbumin from urine ("mouse urine protein") and albumin from skin are important allergen. Urine from female rodents contains lower quantities of allergenic proteins than urine from male rodents. Concentration of airborne allergens depends on the level of proteins in the rodent environment.

After hire, up to one third of workers with extensive exposure to rodents will develop allergy, and up to one tenth will develop asthma. Most workers without prior rodent allergy who become allergic do so during the first 2-3 years after hire. People who have a prior personal history or family history of asthma, hayfever, or eczema will be more likely to develop rhinitis (nasal allergy) and conjunctivitis (eye redness, itching, tearing) than people without such personal or family history.

There is recent evidence that strict limitation of exposure can prevent allergic sensitization and can prevent symptoms in individuals who are already allergic.

Countermeasures for the reduction of mouse skin antigens and airborne allergens include: 1) Engineering controls: separate ventilation systems and negative pressure for cage racks, covering the cages with filter caps, working in fume hoods, and using dust-free bedding to obtain reduction of suspended particulates. Note that high levels of airborne allergens occur in animal rooms, even when animals are undisturbed, especially if such controls have not been implemented. 2) Personal protective equipment: lab coats, disposable gloves, and face masks/respirators-N95, HEPA (high efficiency particulate) or PAPR (positive air pressure respirator). 3) Using only female mice.

If you develop what you believe to be allergic symptoms, report them immediately to your supervisor and visit OMC (Brookhaven's Occupational Medicine Clinic) in Bldg 490.

Appendix 7: IACUC/OB Membership Roster

Please provide a Committee roster, indicating names, degrees, membership role, and affiliation (e.g., Department/Division).

Name of Member	Degree/Credentials	Membership Role	Department/Division
Timothy Green	PhD	Scientist	Environmental Protection Division
DB	AS, RRPT	Non-Scientist	Radiological Control Division
RF	MA	Non-Scientist, Non-affiliated	Unaffiliated
PF	PhD	Scientist	Biology Department
DP	MS, CPG	Non-Scientist	Environmental Protection Division
AS	MS	Non-Scientist, Non-affiliated	Unaffiliated
SS	MS	Non-Scientist	Planning, Performance and Quality Management Office
NS	MBA	Non-Scientist	Environmental Protection Division
TZ	DVM, MPVM, DACLAM	Veterinarian**	Biology Department
PG	PhD	Ex-officio, non-voting	Biology Department
DM	BA	Ex-officio, non-voting	Safety and Health Services Division
MP	BS, RLATG, CMAR, CPIA	Ex-officio, non-voting	Biology Department
CB	BA	Ex-officio, non-voting	Biology Department

Appendix 8: IACUC/OB Meeting Minutes

Minutes of the BNL Institutional Animal Care and Use Committee
May 11, 2020

Present: C. Baran (non-voting), D. Buckallew, R. Feldman, P. Freimuth, T. Green, P. Guida (non-voting), D. Paquette, M. Petry (non-voting), A. Sachs, S. Scocca, N. Sundin, T. Zimmerman

Secretary: D. Mallon (non-voting)

Guests: A. Janczewski, L. Loudenslager, B. Lettieri, A. Rusek

The meeting was held through Blue Jeans and was called to order at 10:00 am by T. Green, Chair, with nine voting members present.

The meeting was held to discuss the proposed Summer run at the NSRL. Five teams were identified that already have animals left over from Spring or that were bred for Summer before the pandemic hit. They are Fornace (345/515), Shay (417), Boerma (507), Porada (500) and Allen (511). The request was to have BLAF staff perform the exposures since no outside users are permitted on site. They will all be whole body exposures with no manipulations. The animals will be exposed at NSRL and shipped back to the home institutions. The research teams will be in contact daily through Blue Jeans or Teams to monitor the studies. A member questioned whether BLAF staff had all the required training to work at NSRL and the response was that they did. Approximately 1,200-1,500 animals will be studied over a 5 week period beginning 6/1/20. The NSRL will run 6 days a week and the animals will be housed at NSRL. The building will be posted as a radiation area so no RCD staff will be required to be in the building. PPE will be used by all personnel as well as social distancing requirements. It was discussed whether BLAF staff would need to be added to each protocol that they were working on. It was agreed that since they are basically just handling the animals as is already part of their duties, they do not need to be added to the protocols. It will be made clear that approval for BLAF staff to perform work usually performed by NASA teams is being given only for the special circumstances surrounding the pandemic. It was questioned if there was any information on whether rodents can have or transfer COVID-19 and the response was that there has been no indication that they could. It was questioned how the Gamma irradiations would be performed and the response was that individual who runs the gamma equipment was already considered essential staff so would be on site to run the experiment. All IACUC members approved the BLAF staff to perform the whole body irradiations for NSRL users for the 2020 Summer run due to the current COVID-19 pandemic.

IACUC Protocol 500 “WFIRM Translational Research Institute for Space Health (TRISH) Program”, PI: C. Porada. Amendment to add 600 MeV/n silicon-28 ions approved by a vote of 9 yes, 0 no.

IACUC Protocol 507 “ γ -Tocotrienol as a countermeasure against high-energy charged particle-induced carcinogenesis”, PI: M. Boerma. Amendment to add a γ -ray exposure group and 22 mice approved by a vote of 9 yes, 0 no.

There being no further business, the meeting adjourned at 10:30 am.

Respectfully submitted,

Appendix 8: IACUC/OB Meeting Minutes

Darcy Mallon

cc: J. Shanklin
R. Tribble

Minutes of the BNL Institutional Animal Care and Use Committee
June 4, 2020

Present: C. Baran (non-voting), D. Buckallew, R. Feldman, T. Green, D. Paquette, M. Petry (non-voting), A. Sachs, S. Scocca, N. Sundin, T. Zimmerman

Absent: P. Freimuth, P. Guida

Secretary: D. Mallon (non-voting)

Guests: A. Janczewski, L. Loudenslager, B. Lettieri

The meeting was held through Blue Jeans and was called to order at 1:30 pm by T. Green, Chair, with eight voting members present.

Old Business

The minutes of the April 2 and May 11, 2020 IACUC meetings were approved as submitted.

Actions Taken Since the Previous Meeting

IACUC Protocol 500 “WFIRM Translational Research Institute for Space Health (TRISH) Program”, PI: C. Porada. Continuing review approved 04/06/20 by the IACUC Chair as authorized at the previous meeting.

IACUC Protocol 516 “Oxidative Stress and the Neuroconsequences of Spaceflight Environment - Immune Dysregulation and Antioxidant Dietary Countermeasure Efficacy”, PI: A. Ronca. Amendment to add 22 mice, clarify euthanasia procedures and terminal blood draw and perfusion methods, minor changes to housing and timing of retro-orbital bleeds and staffing update approved 04/16/20 by the IACUC Chair as authorized at the previous meeting.

IACUC Protocol 501 “Effect of GCR Exposure on the Functional Status of the DNA Repair Machinery and Its Contribution to Cancer Risk”, PI: W. Dynan. Inactivation by PI.

IACUC Protocol 511 “Brain Photomodulation after Whole Body Irradiation”, PI: A. Allen. Request to add UAMS as vendor approved 05/14/20 by Veterinarian Verification and Consultation.

IACUC Protocol 515 “Space radiation-induced persistent estrogenic response and risk of breast cancer development”, PI: A. Fornace. Request to add 100 wild type mice approved 05/14/20 by Veterinarian Verification and Consultation.

New Business

IACUC Protocol 502 “Space Relevant Radiation-Induced Cardiovascular Disease Risk Thresholds: Effect of Gender on the Outcome”, PI: D. Goukassian. Continuing review discussed. It was noted that one of the investigators did not have more than one year of training. The PI will be

Appendix 8: IACUC/OB Meeting Minutes

reminded that the individual must take appropriate training before starting work. Minor typos will be corrected. The IACUC voted 8 yes, 0 no to approve the continuing review for one year effective 07/11/20.

It was noted that the NSRL has begun the limited Summer run. There have been some animal deaths due to fighting. The PI has been notified by BLAF.

There being no further business, the meeting adjourned at 1:55 pm.


Respectfully submitted,

Darcy Mallon

cc: J. Shanklin
R. Tribble

Appendix 9: IACUC/OB Protocol Form

Please attach a **blank** copy of form(s) used by the IACUC/OB to review and approve studies. Include forms used for annual (or other periodic) renewal, modifications, amendments, etc., as applicable.

BROOKHAVEN NATIONAL LABORATORY				
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)				
ANIMAL USE PROTOCOL				
<p><i>The protocol must be typed and all questions must be answered. Answers must be written in English and in lay terms understandable to all IACUC members.</i></p>				
		<table border="1"> <tr> <td>PROTOCOL #</td> <td></td> </tr> </table>	PROTOCOL #	
PROTOCOL #				

Title			
Principal Investigator*			
Institution			
Street Address			
Phone			
Fax			
E-mail			
Key Investigators*			
<small>* Note - if no investigators are BNL employees, please list a BNL employee contact</small>			
Funding Source Submit animal methods section of grant			
Home Institution IACUC Approval # and dates			

Appendix 9: IACUC/OB Protocol Form

A. OVERVIEW
A.1 Please provide a brief description of the proposed studies in lay terms.

Appendix 9: IACUC/OB Protocol Form

B. PERSONNEL AND TRAINING

B.1 In each box, list all personnel working directly with animals and indicate number of years of experience for each procedure for each species.

NAME	SPECIES	MONITORING & HANDLING	NONSURGICAL MANIPULATION	ANESTHESIA, SURGERY	BLOOD COLLECTION	EUTHANASIA

Note: Any personnel with less than one year experience in any of the above categories must take the applicable training listed below.

B.2 Indicate which training courses apply to this protocol. Use A to indicate all personnel or put initials of those required to take the training. All courses are located at <http://www.bnl.gov/training>

Required	COURSE TITLE	PROCEDURES COVERED
A	Basic Overview of Laboratory Animal Care and Use	Overview required by all animal users
A	Regulated Medical Waste Management	Required if regulated medical waste (animal carcasses, needles, syringes) will be generated as a result of the work
The courses below are for BNL employees or guest researchers who have less than one year of training. Guest researchers must have appropriate training from their home institution.		
	Biomethodology of the Mouse	Restraint, handling, identification, sexing, husbandry, behavior of mice
	Biomethodology of the Rat	Restraint, handling, identification, sexing, husbandry, behavior of rats
	Experimental Techniques in Rodents	Injections, blood sampling, oral gavage, euthanasia
	Post-Procedure Care of Mice and Rats: Reducing Pain and Distress	Analgesia, pain & distress recognition and alleviation, post-operative care
	Anesthesia in Laboratory Animals	Anesthesia techniques
	Survival Surgery in Rodents	Aseptic surgical techniques

Appendix 9: IACUC/OB Protocol Form

C. PROCEDURES

C.1 Concisely describe all manipulations and experimental procedures performed on animals.

Everything done to the live animal at BNL must be detailed here, including the timing of shipment to/from BNL. A short description of experimental procedures done at collaborating institutions (if applicable) should be included. Include the end point of the experiment and timing of euthanasia, if applicable. Flow diagrams or charts are helpful.

C.2 Does the work duplicate previous experiments/activities? *If yes, justify.*

D. ANIMAL DESCRIPTION

D.1 Species

D.2 Strain/Breed

D.3 Sex

D.4 Supplier

*If not a commercial vendor, a recent health report (no older than three months) from the animal facility must be submitted to the BLAF Manager **at least two weeks before** the planned experiment or shipment of animals. Please contact the BLAF Manager at (631) 344-3620 to make arrangements for the receipt of the animals. Rodents less than three weeks old must be received with their dams.*

D.6 Justify that the work is appropriate to be done in an animal model. *Indicate why a computer or non-animal model is not a viable alternative.*

D.7 Justify species to be used and why a lower phylogenetic species cannot be used.

D.8 List total number of animals to be used.

Appendix 9: IACUC/OB Protocol Form

D.9 Justify number of animals. Indicate design of study groups and statistical methods and include power calculations. Include steps taken to minimize the number of animals required. Flow diagrams or charts are helpful.

E. PAIN/DISTRESS

E.1 List total number of animals at applicable levels of stress/discomfort

LEVEL A: No Pain or Distress: Animals will be euthanized without any treatments or manipulations; OR animals will undergo irradiation with minimally restricted movement and no anesthesia and without anticipated subsequent effects while at BNL.

Level B: Relieved or momentary pain or distress: Momentary pain or potential pain or distress relieved by pharmacologic, behavioral or other means, e.g., injection of any substance including anesthetics, post-procedural analgesics, behavioral conditioning, restraint or minor pain/distress and medical treatment of disease states.

Level C: Unrelieved or sustained pain or distress: Any procedure that would cause more than momentary or slight pain or distress, e.g., chronic untreated disease states, pain research.

Species	LEVEL A	LEVEL B	LEVEL C

Include scientific justification for any animals in Level C.

E.2 For animals used in Level B or C, perform a literature search for alternatives to pain/distress.

Use this link for search tips: <https://www.nal.usda.gov/awic/awic-tips-searching-alternatives-animal-research-and-testing>

List procedures that may cause pain/distress (e.g. imaging, surgery, injection, behavioral testing, food restriction, etc.) and perform a search using the procedure terms. *Procedures that have pain eliminated by the use of anesthetics and/or analgesics are still considered painful even though the animal is not expected to experience any pain/distress.*

Date of Search	
Databases Searched	

Appendix 9: IACUC/OB Protocol Form

Key Words Searched. <i>Include the word "alternatives"</i>	
Provide a narrative of Search Results. <i>List how many "hits" were obtained for key words used. If alternative procedures are discovered, identify them and justify why those procedures are not being considered.</i>	

E.3 For animals used in Level B or C, indicate how procedures have been refined to reduce the amount of potential pain, distress or morbidity.

E.4 Describe if animals are subjected to food/water deprivation or prolonged and/or unusual restraint and provide justification. <i>Describe how animal health is monitored during deprivation/restraint.</i>

E.5 Is death used as a study endpoint wherein animals must die without intervention such as pain relief and/or euthanasia? <i>If yes, explain why an earlier end point is not acceptable.</i>

F. ANIMAL CARE
F.1 Describe additional requirements for other than routine animal care (e.g. housing, feeding, hazardous waste bedding disposal).

Appendix 9: IACUC/OB Protocol Form

F.2 Scientifically justify if animals must be singly-housed (excluding male mice).

F.3 Scientifically justify if animals will not be provided with environmental enrichment.

G. PROCEDURE SPECIFICS

G.1 List all chemical agents (Sedatives, analgesics, anesthetics, paralytics, euthanasia, study drugs, radiotracers) administered to the animals. *For ketamine anesthesia, please use intraperitoneal (ip) injections, not intramuscular (im). Ketamine/xylazine may be stored for up to 28 days after mixture.*

Type	Agent	Dose	Route	Frequency	Controlled Substance (Y/N) *

*Note: Controlled Substance Awareness training and a DEA background Check will be required for anyone using controlled substances.

For any non-pharmaceutical grade drugs/substances, the preparation and use of the drug must be scientifically necessary, appropriately justified and approved by the IACUC, taking into consideration the side effects, stability, sterility and aseptic handling, storage requirements and other considerations associated with the preparation of this agent. *Please provide information in the box below for all non-pharmaceutical grade substances listed above.*

G.1.a List the name(s) of the individual(s) administering the above agents.

Appendix 9: IACUC/OB Protocol Form

G.1.b If paralytic agents are used in conjunction with surgical manipulations, indicate the means by which absence of pain is monitored and/or determined, and who is responsible.

G.2 Is surgery involved? *If yes, indicate whether surgery is survival or non-survival.*

G.2.a Describe monitoring and supportive care provided during surgery (*who, what and how will this be done?*)

G.2.b Describe indications for analgesic therapy to be administered before, during, and/or following surgery.

G.2.c Describe post-operative and supportive care (*who, what and how often*). **Please use Surgery and Recovery Record ([link to document](#))**

G.2.d Who will maintain surgical and post-operative records and where will they be maintained? *Records must be accessible for inspection and originals for USDA covered species must remain at BNL.*

G.3 Is anesthesia involved?

G.3.a Describe monitoring and supportive care provided during anesthesia (*who, what, and how will this be done?*). **Please use Surgery and Recovery Record ([link to document](#))**

G.3.b Who will maintain anesthetic records and where will they maintained? *Records must be accessible for inspection and originals for USDA covered species must remain at BNL.*

G.4 Are animals to be used in more than one major surgical procedure from which they are allowed to recover? *If yes, please describe and justify.*

Appendix 9: IACUC/OB Protocol Form

G.5 For euthanasia performed at BNL (planned or unplanned), what method and by whom will animals be euthanized and how will death be confirmed? If a chemical agent is used, please list it in Section G.1. For euthanasia involving CO₂, please use 100% CO₂ at a 60% air replacement per minute rate. Justification must be provided for any physical method, such as decapitation, without anesthesia.

G.6 List criteria for intervention and/or removal of animals from study or unplanned euthanasia.

- Examples are severe ataxia, rapidly increased heartrate or respiratory rates, oral, nasal or vaginal discharge such as pus or blood, wound dehiscence, marked swelling, tumor(s) greater than 2 cm or ulcerating, inability to eat or drink or loss of weight greater than 15%.
- Unless otherwise noted, 100% CO₂ at a 60% air replacement per minute rate will be used for early euthanasia for rodents.

H. SPECIAL CONSIDERATIONS

H.1 Check materials used in this study that are hazardous to personnel.

<input type="checkbox"/> Human cells or fluid	<input type="checkbox"/> Microorganism/Virus	<input type="checkbox"/> Chemicals including fixatives	<input type="checkbox"/> Recombinant DNA
<input type="checkbox"/> Nanoparticles	<input type="checkbox"/> Radioactivity (isotopes)	<input type="checkbox"/> Irradiation	<input type="checkbox"/> Other (Specify)

For each agent listed above, please ensure that it is covered under an approved ESR.

H.2 Indicate if animals will be shipped from BNL. If yes, indicate that BNL's preferred shipping procedures will be followed. If other arrangements are necessary, please describe.

H.3 If not shipped from an approved vendor, detail how animals will be transported to BNL.

Appendix 9: IACUC/OB Protocol Form

I. INVESTIGATOR ASSURANCE

I affirm to the best of my knowledge that all the above information is complete and accurate and agree to accept responsibility for this project in accordance with applicable Federal and State of New York regulations, USDA guidelines and established BNL policies and procedures.

No changes will be implemented without approval from the IACUC.

In order to reduce risk to all personnel and laboratory animals, I agree to:

- a. Follow BNL procedures for aspects of the animal care and use such as preoperative care, anesthesia, surgical technique, postoperative care, sampling techniques, euthanasia, and disposal of contaminated carcasses and waste.
- b. Ensure that my instructions to project personnel are implemented.
- c. Ensure that all project personnel complete all required training before handling animals.
- d. Instruct all personnel in my laboratory that they should inform me if they believe that the treatment of any research animal is inappropriate. If the situation is not resolved, the employee should contact the Attending Veterinarian, the IACUC Chair and/or Institutional Official.
- e. Ensure that all research outlined under this protocol shall be carried out under approved Experimental Safety Review(s) (ESR).

PRINCIPAL INVESTIGATOR		DATE	
<i>Your Department Safety Coordinator will be notified of your IACUC approval.</i>			
BNL DEPARTMENT		DATE	

Please review the attached Principal Investigator Roles/Responsibilities/Accountabilities /Authorities and detach the page before submission to the IACUC.

Appendix 9: IACUC/OB Protocol Form

Principal Investigator Roles/Responsibilities/Accountabilities/Authorities

Role

- Design and conduct properly approved research studies involving animals.

Responsibilities

- Know and adhere to federal and BNL rules and regulations governing research involving animals.
- Submit research protocols and addenda to approved protocols to the IACUC for approval prior to start of work.
- Retain copies of all correspondence with the IACUC.
- Submit substantive annual reports of the results of research conducted under IACUC protocols.
- Identify in the IACUC protocol all personnel working on the protocol, including other investigators, technicians, etc.
- Direct the work of staff members conducting research under IACUC protocols.
- Ensure that all protocol personnel are appropriately qualified and that training is kept up to date, including facility-specific training.
- Follow all Brookhaven Laboratory Animal Facility (BLAF) policies and procedures.
- Ensure that animals are properly handled and monitored during a procedure or surgery, as defined in the IACUC approved protocol, and ensure that research procedures that are followed are those defined in the IACUC approved protocol.
- Ensure that animals receive appropriate post-procedure care and monitoring.
- Ensure that animals are transported, on and/or off-site, by proper methods.
- Ensure that all approved protocols, addenda, and terminations are distributed to all protocol personnel, and that such personnel have read and understand the documents.
- Promptly report animal distress or unexpected death to Attending Veterinarian and/or BLAF staff.
- Report any unusual or adverse event or unanticipated problem to the IACUC.
- Maintain records according to Sponsor's requirements or for at least three years following completion of research.
- Make records available for inspection by the IACUC, the United States Department of Agriculture (USDA) or other federal or state government agencies as required.

Accountabilities

- To the IACUC
- To the Department Chair
- To the Department Training Coordinator

Authorities

- Stop or interrupt an animal research study upon evidence of a hazard to the safety of study personnel
- Stop or interrupt an animal research study if an issue of animal welfare and/or protocol compliance arises

BROOKHAVEN NATIONAL LABORATORY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

AMENDMENT

Appendix 9: IACUC/OB Protocol Form

Protocol #:

Protocol Title:	
Principal Investigator:	
A. Description of Proposed Amendment:	
B. Justification and Rationale for Amendment:	
C. Relevance to Original Protocol:	
D. Describe any new painful/distressful procedures, potential adverse effects from the procedures and how the effects will be ameliorated. Verify all personnel are appropriately trained for the procedure(s).	
E. Species, Strain, Number of Animals Requested and Vendor under amendment (if changed):	
F. Justification for Number of Animals in E (include power calculations, where applicable) (if changed):	
G. Method(s) of Anesthesia and/or Euthanasia (if changed):	
H. Will the amendment change the pain or distress or distress category? If yes, please complete the following:	
Date of Search: Database(s) Searched: Keywords Searched: Years Included in Search: Narrative of Search Results:	

I am aware that all research outlined in this amendment must be carried out under approved Experimental Safety Review(s) (ESR) and that this amendment must contain the same information as that listed in the approved ESR(s). I am aware that it is my responsibility to ensure that all individuals working on this protocol have been listed on an appropriate ESR and that their training is up to date.

Appendix 9: IACUC/OB Protocol Form

Principal Investigator's Signature: _____ _____	Date: _____
Department Review: _____ _____	Date: _____

BROOKHAVEN NATIONAL LABORATORY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)



CONTINUING REVIEW FORM

Protocol #:	
Title:	
Principal Investigator:	
Institution:	
BNL Contact:	

In accordance with BNL Policy, the IACUC reviews all research protocols involving animals no less than annually.

The above protocol is approved until . In order for the IACUC to approve this protocol for another year, please return the completed, signed form and any attachments to D. Mallon by . The period of review is .

1. PROTOCOL STATUS: Please indicate (X) the status of this project by clicking on the appropriate box.

Request protocol continuance

Request protocol termination

☐ Active – project ongoing

☐ Inactive – project never started

☐ Not started – anticipated start date:

☐ Completed – no further activities will be done

2. RECORD OF ANIMAL USAGE

Appendix 9: IACUC/OB Protocol Form

Species	Total approved	Used at BNL during reporting period	Pain/Distress Category (A, B, C – see below)	Single housing (Y/N)*

*If Y, please provide scientific justification:

USDA PAIN/DISTRESS CATEGORY

LEVEL A: No Pain or Distress: Animals will be euthanized without any treatments or manipulations; OR animals undergo irradiation with unrestricted movement and no anesthesia and without anticipated subsequent effects while at BNL.

LEVEL B: Relieved or Momentary Pain or Distress: Momentary pain or potential pain or distress relieved by pharmacologic, behavioral or other means. e.g., tranquilization/sedation, general or local anesthesia, post-procedural analgesics, behavioral conditioning to restraint or minor pain/stress, medical treatment of disease states

LEVEL C: Unrelieved or Sustained Pain or Distress: Any procedure that would cause more than momentary or slight pain or distress. e.g., chronic untreated disease states, pain research

3. LITERATURE SEARCH: For animals used in Level B or C, perform a literature search for alternatives to pain/distress.

Use this link for search tips: <https://www.nal.usda.gov/awic/awic-tips-searching-alternatives-animal-research-and-testing>

List procedures that may cause pain/distress (e.g. imaging, surgery, injection, behavioral testing, food restriction, etc) and perform a search using the procedures. *Procedures that have pain eliminated by the use of anesthetics and/or analgesics are still considered painful even though the animal is not expected to experience any pain/distress.*

Appendix 9: IACUC/OB Protocol Form

Date of Search:	
Databases Searched:	
Key Words Searched: : <i>Include the word "alternatives"</i>	
Years Included:	
Provide a narrative of Search Results. <i>List how many "hits" were obtained for key words used. If alternative procedures are discovered, identify them and justify why those procedures are not being considered:</i>	

4. PERSONNEL							
In each box, list all personnel currently working directly with animals and indicate number of years of experience for each procedure for each species. Please use N/A if procedure will not be performed.							
NAME	SPECIES	MONITORING & HANDLING	NONSURGICAL MANIPULATION	ANESTHESIA, SURGERY	BLOOD COLLECTION	EUTHANASIA	

5. PROGRESS REPORT. Please provide a brief but complete, non-scientific description of work done, data collected and conclusions reached, if any, during the past year. If no work has been done, this should be indicated.

Appendix 9: IACUC/OB Protocol Form

6. PROBLEMS/ADVERSE EVENTS. Describe any unanticipated adverse events, morbidity or mortality, the cause(s), if known, and how these problems were resolved. **If NONE, this should be indicated.**

--

7. DUPLICATION. Does the work duplicate previous experiments/activities? If yes, justify.

--

8. FUTURE PLANS. Please provide a brief non-scientific description of what is to be studied during the coming year (the questions/hypotheses being tested in lay terms). If changes are planned, submit an addendum that outlines planned studies and justification for the proposed changes. *[Please note that if the modifications are significant, you may be required to complete a new application. If you have questions or require assistance in making this determination, please contact the IACUC Office and/or the Attending Veterinarian.]*

--

CERTIFICATION OF THE PRINCIPAL INVESTIGATOR I am aware that all research outlined in this protocol must be carried out under an approved Experimental Safety Review (ESR) and that the protocol must contain the same information as that listed in the approved ESR(s). I am aware that it is my responsibility to ensure that all individuals working on this protocol have been listed on the ESR(s), that their training is appropriate and up to date and that they have read and understood their responsibilities on this protocol.

PRINCIPAL INVESTIGATOR		DATE	
<i>Your Department Safety Coordinator will be notified of your IACUC approval.</i>			
DEPARTMENT		DATE	

Appendix 10: IACUC/OB Periodic Program Review and Facility Inspection Report

DATE: March 5, 2020

TO: R. Tribble, Institutional Official

FROM: T. Green, Institutional Animal Care & Use Committee (IACUC) Chair

SUBJECT: **Semi-Annual Report**

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.

All facilities are accredited by AAALAC International.

Evaluation of the Animal Care and Use Program

The IACUC conducted its semiannual evaluation of the Institution's Animal Care and Use Program on February 6, 2020 using the 8th Edition of the *Guide for the Care and Use of Laboratory Animals (Guide)*.

No deficiencies were identified and all aspects of the program are consistent with applicable animal welfare regulations and PHS regulations. Minor wording revisions were made and references to turtle research were removed since this program is no longer active. Additionally, references to USDA were removed and updates made to the Animal Environment, Housing and Management section were made.

Inspection of Animal Facilities

The IACUC inspected the BLAF, the transport van, animal laboratories and the JL Shepherd irradiator (A. Sachs, R. Feldman, D. Buckallew) on February 6, 2020 and the NSRL (T. Green, T. Zimmerman) on February 6, 2020 using the Guide.

There were no deficiencies found.

Minority Views

There were no minority views to this report.

Animal Census

Appendix 10: IACUC/OB Periodic Program Review and Facility Inspection Report

The following is a daily, average census of the animal population for the past six months:

<u>Species</u>	<u>Number</u>
Mice	1182
Rats	80

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Semi-Annual Review of Animal Facilities

<u>Name</u>	<u>Signature</u>	<u>Date</u>
T. Green	_____	
D. Buckallew	_____	
R. Feldman	_____	
P. Freimuth	_____	
D. Paquette	_____	
A. Sachs	_____	
S. Scocca	_____	
N. Sundin	_____	
T. Zimmerman	_____	

PI	Building	Room	Date	Finding(s)	Corrected
Petry	490	Van	08/01/19	None	
Petry	490	BLAF	08/01/19	None	
Guida	490	9-708	08/01/19	None	
		9B-134B		None	
Guida	958	A1, A2, A3	08/01/19	None	

Appendix 10: IACUC/OB Periodic Program Review and Facility Inspection Report

Semi-Annual Report for the Animal Care and Use Program at BNL

08/01/19-02/01/20

Summary of NSRL Run 19C:

The fall NSRL campaign (NSRL 19C) began on September 23, 2019 and ended on November 22, 2019. A total of 26 teams performed experiments at NSRL over this time period, 16 of which utilized animal subjects. There were no deviations from approved IACUC protocols for these teams. The only atypical event was that one research team experienced the unexpected loss of several of their rats, which was immediately reported to the BNL IACUC. The PI and Co-PI also attended an IACUC meeting while on site to describe the situation and provided a write-up of the situation. Potential factors were discussed and conditions were slightly altered. After this initial period, no further loss of their rats occurred.

Post-Approval Monitoring:

Two protocols (329 and 510) were randomly selected for review. Review of training records for personnel listed on the protocols verified training was up to date for all Guests who had been processed to work at BNL. The protocols were also compared to the Experimental Safety Reviews (ESR) to ensure the information was consistent. No discrepancies were discovered.

The following were filed:

AAALAC Annual Report for 2019 (01/20)

USDA Annual Report for 2019 (10/19)

OLAW Annual Report for 2019 (01/20)

DOE Semi-Annual Report (09/19)

NYS Department of Health 2020 Renewal (11/19) – 2020 Certificate received (01/20)

Appendix 11: Heating, Ventilation and Air Conditioning (HVAC) System Summary

Summarize the heating, ventilation and air conditioning (HVAC) systems for each animal facility, **including all satellite facilities**. Include **all animal holding rooms** (including satellite holding rooms), surgical facilities, procedure rooms, and support spaces integral to animal facilities (e.g., cage wash, cage and feed storage areas, necropsy, treatment).

Location/Building/Facility:	
------------------------------------	--

In the text box below, provide a general description of the mechanical systems used to provide temperature, humidity and air pressure control. Include details such as:

- the source(s) of air and air recirculation rates if other than 100% fresh air
- treatment of air (filters, absorbers, *etc.*)
- design features such as centralized chilled water, re-heat coils (steam or hot water), individual room vs. zonal temperature and relative humidity control, the use of variable air volume (VAV) systems and other key features of HVAC systems affecting performance
- features that minimize the potential for adverse consequences to animal well-being (such as re-heat coils that fail closed or that are equipped with high-temperature cut-off systems), and
- how room temperature, ventilation, and critical air pressures are monitored and maintained in the event of a system or component failure, including notifying appropriate personnel in the event of a significant failure that occurs outside of regular working hours and/or other management systems used to respond to alerts or failures.

The animal facility in Bldg. 490 is serviced by three HVAC systems supplying once-through-air at a rate of 10-15 air changes per hour; AC 1 services perimeter rooms such as the lounge, rest room, Feed room, utility room and supply room; AC 2 services all animal rooms, and AC 3 services other areas such as the wash rooms and corridors. These AC units provide positive and negative air control zones for balance so animal rooms remain clean. There is a duct bypass system built-in to enable the use of all three air handlers in any area.

Air is treated through pre-filters, pre-heat coil, centralized chilled water coils, reheat coil and final filters before going into the duct work. Supply air efficiency of filter averages 80-85% as determined by ASHRAE Standard 52 atmospheric dust spot method. Animal rooms are alarmed and provided with individual control of temperature and humidity. Environmental conditions are continuously monitored by an Automated "Logic" Control (ALC) system which can be accessed 24/7 for review. In addition to automated system, table-top hygrometers are used to measure and record temps/humidity in animal rooms daily, high and low values are recorded weekly.

General safety features: The unit shuts down and generates an alarm when the freezestat trips at 37° F. The freezestat is "hardwired interlocked", independent of controller software. The outside damper closes and the fan is de-energized when the

Appendix 11: Heating, Ventilation and Air Conditioning (HVAC) System Summary

freezestat trips, requiring manual reset. If all power or air fail then the preheat valves fail open to protect the air handler unit, and reheats fail closed to protect space. The unit shuts down and sends an alarm if supply air smoke status is detected.

The supply fan runs when set unless it is shut down by safety controls.

The animal facility operations manager is automatically notified through e-mail or phone call when temperature and humidity ranges

fall within a preset abnormal range: Alert: Temp high/low 78°/66°; RH high/low 75%/25%. The Laboratory's Alarms Group is notified when readings reach a second, preset abnormal value: Critical-Temp high/low 80°/60°; RH high/low 80%/20%. These readings trigger an immediate response by HVAC personnel to the affected area. In the event of primary power failure, emergency power is provided to the entire animal facility. This includes all ventilation, heating, and air conditioning systems. In addition to the automated building monitoring system, the ESH Manager measures and documents ventilation rates and pressure gradients annually. Facility and Operations (F& O) Department is instructed to notify on site HVAC mechanics 24/7 in the case of system failures.

The Bldg. 958 NSRL animal area has 3 rooms connected by a common hallway and isolated from rest of the facility by a set of double doors. The Animal Area is serviced by a dedicated HVAC system.

100% outside air is provided to the Animal Rooms by a single self-contained HVAC unit located outside the building. The unit is a DX system with hot gas reheat and an electric heater which provides ~1500 CFM to the Animal Area distribution ductwork.

All supply air is filtered at the inlet of the HVAC unit with a disposable fiberglass filter. The air is further filtered at each supply register before entering a room with disposable filters.

A steam humidifier located in the main supply duct is used to humidify the air as required.

Each room has an independent electric reheat unit and an independent exhaust fan. The exhaust fans are ducted directly to the outside of the building and provide 10-15 air changes per hour.

The temperature and humidity in each room is locally displayed in the hallway outside of each room and continuously monitored by an Automated "Logic" Control (ALC) system which can be accessed online 24/7 for review. Conditions outside the allowable set points are alarmed to the BNL Site Supervisor and the BLAF personnel are notified.

During operations, table-top thermometers/hygrometers are used to daily measure and record temps/humidity in each room, with high and low values recorded weekly.

The ALC System is used as the primary controller for all of the functions of the HVAC system – maintaining the proper temperature and humidity in each room.

Appendix 11: Heating, Ventilation and Air Conditioning (HVAC) System Summary

In the Table below, provide room-specific information requested. Include all animal holding rooms (including satellite holding rooms), surgical facilities, procedure rooms, and support spaces integral to animal facilities (e.g., cage wash, cage and feed storage areas, necropsy, treatment). For each of these rooms/areas, indicate use, including the species for animal housing rooms. *Measurement of air exchange rates and verification of relative pressure within the areas mentioned above must be completed **within the 12 months preceding completion of this Program Description**.* Air exchange rates may be important to maintain air quality in other areas; *however, measurements may be left at the discretion of the institution (e.g., air measurements in areas where aquatics are held.)* Information may be provided in another format, providing all requested data is included. **[Note: Please remove the examples provided in the Table below.]**

Room No.	Specific Use	Temperature Set-Point (define units)	Electronic / Emergency Monitoring of Temperatures (Y/N)	Alert/Alarm Temperature Ranges (if applicable; define units)	Humidity Control (Y/N)	Relative Pressure	Air Exchange Rate (per hour)	Date Verified / Measured
		(settings to be verified)					(values to be measured)	
11-105	Cage Wash-Dirty Side	72°F	N	NA	N	–	13	1/20
11-118	Cage Dumping Station	72°F	N	NA	N	–	13	1/20
11-143	Food Storage	65°F	N	NA	N	–	14	1/20
11-147A	Procedure Room	72°F	Y	NA	Y	+	15	1/20
11-147B	Rodent Housing	72°F	Y	70-74°F (alert) critical 66-78°F	Y	+	14	1/20
11-147C	Mouse IVC Housing	72°F	Y	70-74°F (alert) critical 66-78°F	Y	+	14	1/20
11-205	Cage Wash-Clean	72°F	N	NA	N	+	13	1/20
11-205A	Clean Storage/Bedding	72°F	N	NA	N	+	15	1/20

Appendix 11: Heating, Ventilation and Air Conditioning (HVAC) System Summary

Room No.	Specific Use	Temperature Set-Point (define units)	Electronic / Emergency Monitoring of Temperatures (Y/N)	Alert/Alarm Temperature Ranges (if applicable; define units)	Humidity Control (Y/N)	Relative Pressure	Air Exchange Rate (per hour)	Date Verified / Measured
		(settings to be verified)					(values to be measured)	
11-205B	Supply Storage	70°F	N	NA	N	+	15	1/20
11-241	Rodent Housing Normal Rodent Hindlimb Unloading	72°F 74-78 °F	Y	70-74°F (alert) Critical alarm low 66-78°F 72 alert critical alarm- low-70, high 82	Y	—	14	1/20
11-242	Rodent Housing	72°F	Y	70-74°F (alert) critical 66- 78°F	Y	—	15	1/20
A-1 NSRL	Rodent Housing	72°F	Y	70-74°F (alert) critical 66- 78°F	Y	—	13.5	1/20
A-2 NSRL	Mouse IVC	72°F	Y	70-74°F (alert) critical 66- 78°F	Y	+	14.0	1/20
A-3 NSRL	Rodent Housing/Utility	72°F	Y	70-74°F (alert) critical 66- 78°F	Y	+	13.6	1/20

Copy and repeat the Description and Table for each location, including all satellite housing locations.

Appendix 12: Aquatic Systems Summary – Part I

Please summarize water management and monitoring information programs for each animal facility, including all satellite facilities, rooms, cephalopod housing systems, and enclosures. The following key will assist you in completing the form:

- (1) List location of aquaria, including outdoor enclosures (ponds or outdoor tanks). If indoors, list building and room number.
Note that all species housed at the same location and maintained via the same design and monitoring may be listed in the same row.
- (2) Please indicate if embryonic (E), larval (L), juvenile (J) or Adult (A)
- (3) Group tanks (ponds, outdoor tanks, multiple aquaria) are arranged as arrays with shared water supply; individual aquaria have exclusive water handling systems.
- (4) Indicate water type, e.g., fresh, brackish, or marine.
- (5) Indicate water pre-treatment, e.g., dechlorination, rough filters.
- (6) Indicate water circulation, e.g., static, re-circulated, constant flow, or some combination of these. If applicable, indicate water exchange frequency and amount (percentage).
- (7) Provide a key word for filtration employed, e.g., biological, chemical, mechanical, and type (e.g., mechanical-bead filter).
A diagram may be provided showing the flow of water, filtration, source of “make-up” water and amount replaced daily.

Part I

Location (1)	Species (2)	System Design					
		Group / Individual (3)	Water Type (4)	Pre-treatment (5)	Circulation (6)	Filtration (7)	Disinfection (e.g., UV, ozone)

Note: Records of equipment maintenance (filter changes, UV bulb changes, probe changes, calibrations, *etc.*) should be available for review.

[Create additional rows by pressing TAB in the bottom-right box.]

Appendix 12: Aquatic Systems Summary – Part II

The following key will assist you in completing this form:

- (1) In these columns, please indicate monitoring frequency, e.g. daily, weekly, monthly or other point sampling frequency; continuous/real time, or none, if applicable. Also indicate method of control (heaters versus room HVAC, hand versus auto dosing, etc.).
- (2) Indicate other parameters and their monitoring frequency, e.g., alkalinity, total hardness, conductivity, chlorine/chloramine.

Part II

Monitoring									
<i>Indicate in the boxes below the frequency of monitoring and method of control for the following parameters. (1)</i>									
Location (from Part I)	Temperature	Salinity	pH	NH ₄	NO ₂	NO ₃	Dissolved O ₂	Total Dissolved Gases	Other. Please List (2):

Note: This information may be provided in another format, provided that all requested data is included.

[Create additional rows by pressing TAB in the bottom-right box.]

Appendix 13: Primary Enclosures and Animal Space Provisions

Please complete the Table below considering performance criteria and guiding documents (e.g., *Guide*, *Ag Guide*, ETS 123 and/or other applicable standards) used by the IACUC/OB to establish adequacy of space provided for all research animals including traditional laboratory species, agricultural animals, aquatic species, and wildlife when reviewing biomedical, field, and agricultural research studies. Refer to AAALAC International's Position Statement ["Cage or Pen Space"](#) for additional guidance.

Species	Dimensions of Enclosure (cage, pen, tank*, corral, paddock, etc.)	Maximum Number Animals / Enclosure	Guiding Document Used to determine the Institution's Space Standards (<i>Guide</i> , <i>Ag Guide</i> , ETS 123, Other)	Enclosure Composition & Description**
RAT	CAGE 10 1/2 x 19 x 8	2	Guide	High temp, autoclavable Polycarbonate with micro-isolator lid
MOUSE	IVCS cage 11 1/4 x 6 7/8 x 5	5	Guide	High temp, autoclavable Polysulphone with micro-filter top
MOUSE	CAGE 10 1/2 x 6 1/2 x 5	4 >25 grams 5 <25 grams	Guide	High temp, autoclavable Polycarbonate with micro-isolator lid
MOUSE	CAGE 10 1/2 x 19 x 6 1/8	10	Guide	High temp, autoclavable Polycarbonate with micro-isolator lid

*For aquatic species, provide tank volume.

**Include descriptors such as open-topped, static microisolator, individually-ventilated cage systems (IVCS).

Appendix 14: Cleaning and Disinfection of the Micro- and Macro-Environment

Please describe the cleaning and disinfection methods in the Table below. Note the washing/sanitizing frequency and method for each of the following:

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Micro-environment				
Solid-bottom cages (static)	Tunnel washer	1-2 week	Enviro-Kleen 1200 & Quiptrol 3000	
Solid-bottom cages (IVC)	Tunnel washer	1-2 week	Enviro-Kleen 1200 & Quiptrol 3000	
Suspended wire-bottom or slotted floor cages	N/A	N/A	N/A	
Cage lids	Tunnel washer	Every two weeks	Enviro-Kleen 1200 & Quiptrol 3000	
Filter tops	Tunnel washer	Every two weeks	Enviro-Kleen 1200 & Quiptrol 3000	
Cage racks and shelves	Rack washer	Monthly IVC Racks are disinfected between use	Enviro-Kleen 1200	
Cage pans under suspended cages	N/A	N/A	N/A	
Play pens, floor pens, stalls, etc.	N/A	N/A	N/A	
Corrals for primates or outdoor paddocks for livestock	N/A	N/A	N/A	
Aquatic, amphibian, and reptile tanks and enclosures	N/A	N/A	N/A	

Appendix 14: Cleaning and Disinfection of the Micro- and Macro-Environment

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Feeders	Tunnel washer	Every two weeks	Enviro-Kleen 1200 & Quiptrol 3000	
Watering devices	Tunnel washer	weekly	Enviro-Kleen 1200 & Quiptrol 3000	
Exercise devices and manipulanda used in environmental enrichment programs, etc.	Tunnel washer	weekly	Enviro-Kleen 1200 & Quiptrol 3000	
Transport cages	N/A	N/A	N/A	
Operant conditioning & recording chambers, mechanical restraint devices (chairs, slings, etc.)	Tunnel washer	After each use	Enviro-Kleen 1200 & Quiptrol 3000	
Euthanasia chambers	Tunnel washer	After each use	Enviro-Kleen 1200 & Quiptrol 3000	
Macro-Environment				
Animal Housing Rooms:				
Floors	Pressure washer Mop	Six months as vacated 1 X week	Saniplex 128 M	At least every six months for long-term rodents or after each study
Walls	Pressure washer	Six months as vacated	Saniplex 128 M	At least every six months for long-term rodents or after each study

Appendix 14: Cleaning and Disinfection of the Micro- and Macro-Environment

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Ceilings	Sponge mop/brush	Six months as vacated	Saniplex 128 M	Or as needed
Ducts/Pipes	Sponge mop/brush	Six months as vacated	Saniplex 128 M	Or as needed
Fixtures	Hand wash	Six months as vacated	Saniplex 128 M	Or as needed
Corridors:				
Floors	Strip/Buf Mop	Every three months 1 x week	Buckeye Ripsaw Saniplex 128 M	Floors are mopped at least once weekly or as needed
Walls	Pressure washer	1 X year	Saniplex 128 M	
Ceilings	Brushed/vacuumed	1 X year	Saniplex 128 M	
Ducts/Pipes	Sponge mop /vacuum	1 X year	Saniplex 128 M	
Fixtures	Hand washed	1 X year	Saniplex 128 M	
Support Areas (e.g., surgery, procedure rooms, etc.); complete for each area:				
Floors	Pressure washer Mop	Varied after each use	Saniplex 128 M	After each study run (6-15 weeks)
Walls	Pressure washer	Varied	Saniplex 128 M	After each study run (6-15 weeks)
Ceilings	Sponge mop/brush	Varied	Saniplex 128 M	After each study run (6-15 weeks)
Ducts/Pipes	Sponge mop/brush	Varied	Saniplex 128 M	After each study run (6-15 weeks)
Fixtures	Hand washed	Varied	Saniplex 128 M	
Implements (note whether or not shared):				

Appendix 14: Cleaning and Disinfection of the Micro- and Macro-Environment

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Mops	Rack washer	As needed	Enviro-Kleen 1200	Not shared between species or rooms
Mop buckets	Rack washer	After each use	Enviro-Kleen 1200	Not shared between species or rooms
Aquaria nets	N/A	N/A	N/A	
Other				
Other:				
Vehicle(s)	Hand wash/brush/ vacuum	As needed between species	Saniplex 128 M	
Other transport equipment (list)	N/A	N/A	N/A	

*Please provide chemical, not trade name.

Appendix 15: Facilities and Equipment for Sanitizing Materials

In the Tables below, summarize the facilities and equipment used to sanitize animal related equipment (tunnel washer, bottle washer, rack washer, bulk autoclave, hand washing area, bedding dispensing unit, *etc.*). Note that some descriptions may be combined if all share identical features (e.g., all rack washers).

Building	Room No.	Equipment Type	Safety Feature(s)	Methods of Monitoring Effectiveness
490	11-105	Rack washer	Emergency “off” button; labeled doors-instructional signage “push to open”; no locking mechanism.	Guaranteed 180-degree hot water rinse temp-tape strips used weekly; Difco Hycheck plates of sanitized caging and accessories tested 2-4 times per year by Quip Laboratories, Wilmington, DE. ATP-based luminescence swabs performed at least 1 load/day.
490	11-105	Tunnel washer	Emergency “off” button; extension table for on/off loading cages; plastic curtains at entry and exit to retain hot water inside machine; door sensors on hot water tank doors	Chemical sanitation: Chemical shut-off switch on chemical dispenser. Heat sanitation: Guarantee 180-degree hot water rinse through visual readout and thermocouple shuts off the conveyor belt if temp falls below set-point; Difco Hycheck plating of sanitized caging and accessories tested 2-4 times per year by Quip Laboratories, Wilmington, DE. ATP-based luminescence swabs performed at least 1 load/day.
490	11-205	Bulk autoclave	Emergency “off” button; lock-out key	Autoclave tape and Attest™ – 3M bacterial monitoring system used with each load to verify adequate sterilization.
490	11-205	Bedding dispensing unit	Emergency “off” button	N/A

Appendix 16: Lighting Summary

Using the Table below, summarize the lighting system(s) for the animal housing facility(ies). For each species or holding room type, list light intensity (range), construction features (e.g., water resistance), photoperiod (light:dark) and control (e.g., automatic versus manual, phasing). For systems automatically controlling photoperiod, describe override mechanisms (including alarms, if applicable).

Location:	Bldgs. 490 & 758
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Room Type ^(a)	Light Intensity Range	Lighting Fixture Construction Features ^(b)	Photo-period (hrs) ^(c)	Photoperiod and Lighting Control	Override Mechanisms (if applicable)
Rodent Holding Rooms -490	280-398 lux	Surface mounted, water resistant	12:12	Automatic via wall mounted timer control box	Mechanical on/off switch
Rodent Holding Rooms-958	108-270 lux	Surface mounted, waterproof (sealed)	12:12	Automatic via wall mounted control box	Mechanical on/off switch
Cage-Washing Room	Not measured	Surface-mounted, water resistant.	NA	N/A	N/A

^(a) A list of each room is not needed; group or cluster rooms by species or function

^(b) Include such features as water resistance, red lighting, etc.

^(c) Note if light cycle inverted/reversed.

Repeat Location and Table as necessary for each location, including satellite housing locations.

Appendix 17: Satellite Housing Facilities

Note: In the Program Description Section 2. IV. (Physical Plant), item C., describe the criteria used to determine a “Satellite Animal Holding Area.” In the Table below, summarize these animal housing areas. Note that the total square footage for all each of these must also be included in the Summary of Animal Housing and Support Sites (**Appendix 2**), and applicable information regarding these areas included in the Heating, Ventilation, and Air Conditioning (HVAC) Summary (**Appendix 11**) and Lighting Systems Summary (**Appendix 16**).

Building	Room(s)	Person Responsible	Species Used	Approximate Area (ft ² or m ²) Devoted to Housing	Maximum Period of Stay	Purpose / Rationale / Justification	Construction Features and Finishes
958	A1	M. Petry	Mouse	177 sq. ft.	As needed	Animal holding	”Stonhard” seamless flooring, block, epoxy painted walls and smooth ceilings are moisture resistant; floors sloped to floor drains; sinks; waterproof lighting fixtures
958	A2	M. Petry	Mouse/Rat	241 sq. ft.	As needed	Animal holding	Same as above
958	A3	M. Petry	Multiple use room	160 sq. ft.	2-8 hrs.	Temp animal holding/utility	Same as above

Appendix 17: Satellite Housing Facilities

Please describe below the oversight of cephalopods (for guidance, refer to AAALAC International's Frequently Asked Question, "[Invertebrate animals](#)" and [AAALAC's Reference Resource, "Guidelines for the Care and Welfare of Cephalopods in Research-A consensus based on an initiative by CephRes, FELASA and the Boyd Group,"](#) (Note AAALAC International's caveats regarding this resource). In addition, the care and use of cephalopods may be described in the relevant sections (i.e., housing, husbandry, veterinary care, surgery and euthanasia, etc.) within the Program Description.

N/A