Program Description
Animal Care and Use Program

National Institutes of Health

National Institute on Aging

Intramural Research Program

Redacted by agreement

Baltimore, Maryland 21224

April 01, 2019

For
AAALAC International

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Appendices

Program Description

Instructions for Completing and Submitting the Program Description for the Institutional Animal Care and Use Program

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.

Comparative Medicine Section (CMS)
National Institute on Aging (NIA)
Intramural Research Program (IRP)
National Institutes of Health (NIH)
Public Health Service (PHS)
Department of Health and Human Services (DHHS)

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.

The NIA IRP supports a broad-based research program that seeks to elucidate the biology of aging and age-associated diseases and disabilities. Investigations on the general biology of aging focus on characterizing normal aging and distinguishing between normal and pathological changes with aging. The animal care and use program is a vital part of this research, supporting animal models of Alzheimer's disease; Huntington's disease; Parkinson's Disease; cancer; frailty; cardiovascular disease and hypertension (including age-dependent salt sensitivity); diabetes; and translational research including the development and testing of various intervention strategies to treat age-associated diseases. A significant part of the program consists of efforts to generate and investigate genetic, surgical and other experimental models of aging and disease. The program includes the breeding of genetically engineered mice as tools for these research endeavors.

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.

The NIA IRP maintains an assurance with the Office of Laboratory Animal Welfare (OLAW) via the Office of Animal Care and Use (OACU) of the NIH. The NIA IRP complies with standards of the Guide for the Care and Use of Laboratory Animals (Guide), NRC, 2011, and the PHS Policy on Humane Care and Use of Laboratory Animals, USDHHS, NIH, OLAW, 2015, for all animals, as well as the Animal Welfare Act, USDA, regulations, and USDA Animal Care Policies for USDA's Animal Welfare Act, USDA, APHIS, 1966 (as amended), for these documents' covered species (henceforth referred to as USDA-covered species).

The NIH Policy Manual (PM) 3040-2: Animal Care and Use in the Intramural Program, establishes responsibility for humane care and use of animals within the NIH Intramural Research Program and is applicable to all NIH-conducted or -supported intramural activities involving animals. This policy manual ensures animal program compliance with the provisions of NIH's intramural Institutional Assurance (A4149-01).

The NIA also complies with the NIH Guidelines for *Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, as applicable, *Occupational Health and Safety in the Care and Use of Research Animals*; *Occupational Health and Safety in the Care and Use of Nonhuman Primates* (although we currently do not hold or house any nonhuman primates).

The NIH Animal Research Advisory Committee (ARAC) advises the Institutional Official (IO) regarding interpretation of external regulations and policy as well as develops policies and guidelines to establish best practices and ensure consistency throughout the NIH. The Chair of each ACUC within the NIH is a member of this Committee. ARAC/NIH Guidelines and NIH Policy Manuals are referenced often within the context of our program because they form the foundation of regulatory interpretation at the NIH.

While the general expectation is that these guidelines will be followed as written, any Institute/Center (IC) Animal Care and Use Committee (ACUC) within the NIH may grant departures from ARAC Guidelines based on the specific requirements of an animal study proposal (ASP), when appropriately justified. These NIH Policy Manuals and ARAC Guidelines can be accessed through the OACU website (http://oacu.od.nih.gov/). Hard copies of these documents will be provided for reference on site.

The NIA ACUC also utilizes site- and research discipline--specific standard operating procedures and technical appendices that will be available on site.

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 publicly 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.

Dr. Michael Gottesman, NIH Deputy Director for Intramural Research (DDIR), is the Institutional Official (IO) and has delegated authority and responsibility from the NIH Director and CEO.

Redacted by agreement This position has delegated authority from the IO to oversee and ensure compliance of the NIH IRP with all controlling standards, regulations and guidelines. The position will be advertised in the near future.

Redacted by agreement has delegated authority from the IO through Redacted by agreement to appoint members to the NIA IRP Animal Care and Use Committee (ACUC) and otherwise serves as the IO's on-site representative.

Dr. Jeffrey Long serves as the ACUC Chair.

Redacted by agreement serves as the ACUC Vice Chair.

Dr. Kathy Perdue, the Animal Program Director (APD) and Attending Veterinarian (AV) for USDA purposes, is a Public Health Service Officer (Government employee) and receives delegated program authority from the SD. The APD is also the Contracting Officer's Representative (COR) for the Charles River Laboratories (CRL) contract.

Redacted by agreement is the Redacted by agreement Redacted by agreement She is supervised by the Redacted by agreement and

receives delegated authority from the APD to serve as Veterinarian on the ACUC (to include AV protocol signatory authority).

Redacted by agreement

Redacted by agreement is supervised by the Redacted by agreement and leads provision of veterinary clinical care for all species.

Redacted by agreement is a Government employee supervised by the APD.

Redacted by agreement is a Government employee supervised by the APD and is the Alternate COR for the contract.

Redacted by agreement supervises the CRL contract which provides animal care, veterinary, and other technical services.

Redacted by agreement assists in the supervision of CRL staff and is supervised by Redacted by agreement

An organizational chart, including lines of authority, is provided as Appendix 4.

See Section 2, Paragraph 1.A.2.a.i. . for credentials of veterinary personnel.

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.

NIH Deputy Director for Intramural Research (Institutional Official)	Michael Gottesman, M.D.
Redacted by agreement	Redacted by agreement
ACUC Chair	Jeffrey Long, Ph.D.
Redacted by agreement	Redacted by agreement
ACUC Members	(See Appendix 6)
Animal Program Director (APD)	Kathy Perdue, D.V.M., DACLAM
Redacted by agreement	Redacted by agreement

Redacted by agreement		Redacted by agreement

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.

The NIA intramural program currently has 30 Principal Investigators (PIs) conducting research on 73 approved Animal Study Proposals (ASPs).

The major types of research conducted focus on aging and age-related diseases. The research consists of studies of physiological changes over an entire lifespan, including quantification of age changes, elucidation of mechanisms underlying these changes and the relationship between aging and specific disease states; applying behavioral methods and principles in the assessment, control, and treatment of age-related disorders; original research to describe the influence of age and age-related chronic pathologic conditions on brain and cardiovascular and myocardial function in man, in different experimental animal models in vivo, and in cardiac muscle, myocytes, nerve cells and sub-cellular organelles in animal models; research in molecular genetics to examine age-related alterations in cellular function, and to identify, isolate, and characterize genes involved in aging processes and in age-dependent disorders; studies of the mechanisms of transport processes, the regulation of these systems by hormones and pharmacological agents, signal-transduction mechanisms, and the changes that occur in aging and in age-associated disorders; research into the mechanism of RNA synthesis, as well as hormone therapy, age changes in molecular dynamics and non-invasive magnetic resonance studies; investigations of the structure, functions, physiology, biochemistry, and pharmacology of central and peripheral nervous systems, and other systems including the cardiovascular, renal, endocrine, and skeletal-muscle systems, and investigations of the changes that take place during development and aging; age associated changes in immune function as well as studies targeted toward vaccine development. Some studies are part of translational, bench-to-bedside studies, starting at the cellular level and expanding to experimental animal models with eventual extension to clinical testing in people.

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

The NIA animal research program, as a Federal entity, is supported by federal funds and NIH administered Cooperative Research and Development Agreement (CRADA) 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.

Under the following circumstances the NIA holds animals at the Redacted by agreement Redacted by agreement The facility is AAALAC-accredited (Unit 000777) and managed by the Department of Veterinary Resources (DVR). The NIA ACUC extends their semiannual program review and facilities inspections to include the Redacted by agreement

- Through a Memo-of-Understanding, the Laboratory of Behavioral Neuroscience (LBN) and the Office of the Scientific Director (OSD) house nonhuman primates at the Redacted by agreement
- Imported rodents may also be held at the Redacted by agreement at the Redacted by agreement The Redacted by agreement program assumes full responsibility for animal care of imported rodents [Note: this service is being moved to DVR, Bethesda, MD, in March 2019].

Under the following circumstances, the NIA holds animals in Redacted by agreement Redacted by agreement on the NIH main campus in Bethesda, Maryland. The facility is AAALAC-accredited (Unit 000777) and managed by National Institute of Neurological Disorders and Stroke (NINDS). The NIA ACUC extends their semiannual program review and facilities inspections to include Redacted by agreement

- Through a Memo-of-Understanding, the Laboratory of Neurogenetics (LNG) and the Laboratory of Genetics and Gerontology (LGG) house rodents in Redacted by agreement

Occasionally, the NIA also holds animals in Redacted by agreement on the NIH main campus in Bethesda, Maryland. The facility is AAALAC-accredited (Unit 000777) and managed by the Department of Veterinary Resources (DVR). This housing location is only used for animals with potential infectious concerns for the NIA facility.

These collaborations follow the standards set by OLAW, the Guide, and the ARAC "Guidelines for Collaborative Animal Studies" and "Guidelines for ACUC Oversight of Animal Activities in Shared and Central Facilities". These guidelines mandate such semiannual reviews, as well as monitoring of NIA investigator activities at these shared/central facilities by the APD. The APD and/or Facility or Regulatory Veterinarian also attend periodic clinical rounds at these locations.

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.

N/A

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

N/A

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.

Dr. Jeffrey Long, ACUC Chair, does have the option to communicate directly with the Institutional Official, Dr. Michael M. Gottesman, Deputy Director for Intramural Research. However, due to the immense size of the NIH most interactions related to his responsibilities are conducted with the Director, OACU, or Redacted by agreement who himself has twice-a-month meetings with the IO, Dr. Gottesman. Dr. Long is a member of the NIH Animal Research Advisory Committee (ARAC), a committee comprised of the NIH ACUC chairs and others, which is advisory to Dr. Gottesman. Dr. Long reports to Redacted by agreement Dr. Long submits the Institute's semiannual report of research facilities inspection and animal program review and the USDA Annual report to both Dr. Gottesman and Redacted by agreement through the NIH Office of Animal Care and Use (OACU).

Redacted by agreement currently performs the duties of the Redacted by agreement This position plays a central role in communications between NIA and the NIH IO, Dr. Gottesman. The DDIR/IO has delegated authority to the Director, OACU for ensuring compliance of the intramural ACU program with all laws, regulations, policies and guidance, in order to maintain both PHS Animal Welfare Assurance and AAALAC accreditation. The Director, OACU forwards all pertinent and critical reports and documents for the DDIR/IO's review and acknowledgement/approval: e.g., semiannual reports from all ICs; annual reports to OLAW, AAALAC, USDA; OLAW-reportable events summaries and actions; ARAC-recommended policy changes; recommended policy manual changes, etc. The Director, OACU meets regularly with the DDIR/IO and keeps him apprised of general issues occurring within the IRP animal care and use program.

Redacted by agreement or other NIH OACU members may attend NIA ACUC meetings as observers and may accompany the ACUC on semiannual facility inspections. The OACU reviews all official documents related to the NIA animal care and use program (ACUC semiannual reports, OLAW and

USDA reports, AAALAC correspondence, etc.) and has access to all ACUC agenda materials prior to meetings.

Dr. Kathy Perdue, Animal Program Director (APD) and Attending Veterinarian (AV), reports directly to Redacted by agreement who has delegated authority from the IO to oversee animal care and use activity issues, with certain limitations. She chairs quarterly meetings with senior NIA scientists to discuss the NIA research program and animal program needs. Animal care and use program issues are communicated regularly to the ACUC Chair in monthly ACUC meetings and by frequent direct communication.

Redacted by agreement

serves as the Redacted by agreement for the ACUC and as the regulatory laboratory animal medicine point of contact for all animal care and use activities. She communicates frequently and directly with the ACUC Chair, and on an almost daily basis with the APD; she may communicate with the Director, OACU, as required, regarding regulatory questions.

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.

Kathy Perdue, D.V.M., DACLAM is a PHS Officer/full-time Government employee and devotes 100% of her time to veterinary support of the animal care and use program as the Animal Program Director (APD). She reports to and receives delegated program authority from the Scientific Director (from the Institutional Official) for all activities involving animals and is responsible for ensuring compliance with all applicable regulations, guidelines, and policies (as described above, Section 1.c.). Dr. Perdue:

- Serves as the AWR Attending Veterinarian,
- Oversees the animal care/technical services contract, which provides various services for the animal care and use program. The

Regulatory and Clinical Veterinarians are employed through this contract.

- Reports animal care and use issues to the ACUC, OACU, and Redacted by agreement Redacted by agreement

Redacted by agreement is a (b)(6) CRL employee. Redacted by agreement Redacted by agreement

- Spends approximately (b)(6) of her time in direct support of the animal care and use program from a regulatory standpoint.
- Serves as the Redacted by agreement member for the ACUC. In this role she communicates directly with the ACUC Chair and the APD.
- Provides guidance to investigators on addressing pain and distress in their animal study proposals, as well as all other regulatory components of protocol development,
- Provides regulatory input/review of ACUC-approved research protocol Standard Operating Procedures (SOPs) and appendices.
- Devotes approximately (b)(6) of her time to clinical care by providing clinical care if the Clinical Veterinarian is unavailable, providing primary clinical care for all ABSL-2 animals, and serving on-call on a rotating basis.

Redacted by agreement is a (b)(6) CRL employee. Redacted by agreement

- Provides clinical care.
- Assists with writing CMS animal care SOPs.
- Provides after-hour emergency care.
- Assists in model development or refinements in a clinical/hands-on capacity, as requested

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.

Redacted by agreement on the CRL contract, provides oversight and training to the veterinary technical staff. She is the primary trainer in animal-related activities for all CRL contract staff.

Redacted by agreement on the CRL contract, provides technical support. She participates in the disease detection and

surveillance/diagnosis and treatment program and coordinates sentinel animal testing.

Redacted by agreement on the CRL contact, provides backup technical support as necessary.

Employees hired under the CRL contract as either an Redacted by agreement Redacted by agreement and Redacted by agreement Redacted by agreement or as a Redacted by agreement Redacted by agreement perform animal husbandry and assist in technical support.

Redacted by agreement hired under a Kelly Services contract, is dedicated to the Translational Gerontology Branch (TGB) and performs animal husbandry and technical support.

Redacted by agreement hired under a Kelly Services contract, is dedicated to the Translational Gerontology Branch (TGB) and performs animal husbandry and technical support.

All listed individuals perform animal treatments as prescribed by CMS veterinarians (the Facility or Regulatory Veterinarian).

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.

Only NIA-owned animals are housed in the NIA animal facility of the Redacted by agreement

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These collaborations follow the standards set by OLAW, the Guide, and the ARAC "Guidelines for Collaborative Animal Studies" and "Guidelines for ACUC Oversight of Animal Activities in Shared and Central Facilities". These guidelines mandate such semiannual reviews, as well as monitoring of NIA investigator activities at these shared/central facilities by the APD. The APD and/or Facility or Regulatory Veterinarian also attend periodic clinical rounds at these locations.

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.

NIH PM 3040-2, Section F13.B.15. (ACUC RESPONSIBILITIES) requires that the ACUC oversees the NIA's training program and ensures the program's effectiveness by:

- Tracking all PI and animal users' completion of mandatory training (see requirements, below) and verifying with PIs that the animal users are experienced or have received training on technical, hands-on procedures they will be responsible for conducting.
- Verifying that all animal care staff are experienced or have received training on technical, hands-on procedures they will be responsible for performing.
- Identifying training needs for intramural staff who work with laboratory animals, communicate those needs to the Associate Director for Training, OACU, and assist with the development of the appropriate

courses as requested; however, it is often logistically unfeasible to send NIA investigators to main campus for these courses, so this is done in only a limited manner.
- Advising the SD regarding the requirements for training of professional and technical staff in animal care and use.

The NIA ACUC also:

- Reviews animal user training during semiannual program reviews, or when otherwise indicated if an adverse event or PI request occurs.
- Directs AV or CMS veterinarians to observe individuals whose competencies may be in question, or who request retraining.

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.

Name & Credentials	Qualifications, Training, Continuing Education
Kathy Perdue, D.V.M., DACLAM *Animal Program Director*	Washington State University, D.V.M. 1983; Board Certified ACLAM 2000; CE: NCAB and National AALAS, ACLAM Forum, OLAW & AAALAC Webinars; DC Academy of Veterinary Medicine seminars; Bio conference Live - Lab Animal Sciences Virtual Conferences
Redacted by agreement	

Redacted by agreement

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

1) Indicate the number of animal care personnel.

Redacted by agreement

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.

Certifications	Number of staff	Animal Care Experience	Number of staff
AALAS ALAT	Redacted by agreement	0-2 years	Redacted by agreement
AALAS LAT		3-5 years	
AALAS LATG		5-10 years	
AAALAS ILAM		> 10 years	
AAALAS CMAR			
RVT			

All staff completes mandatory NIH animal training and the mandatory CMS Animal Users Orientation and Basic Animal Handling training course.

As needed, the contractor conducts in-house classes on animal biosafety levels, personal protective equipment, safety and ergonomics, and specific methods and procedures as per CMS SOPs. The CMS arranges training with vendors on the safe use of toxic agents such as chemicals and disinfectants, as needed. The contractor provides weekly animal handling and techniques workshops that are open to investigators and applicable CMS staff. CMS veterinarians provide training on recognizing animal pain and distress.

The investigative staff provides an introduction and training to CMS at the initiation of novel ABSL2 work. Investigators doing ABSL1 work are encouraged to provide pre-study presentations; CMS may request an investigator presentation for complicated studies in which CMS staff are involved.

The animal care contractor conducts training classes to prepare animal care personnel for examination at the three levels of AALAS certification. This training is provided by offering lunchtime seminars and webinars on various topics, and by enrolling employees in other local facility training courses. Animal care personnel are encouraged to attend the annual National Capital Area Branch (NCAB) AALAS seminar and technician workshops.

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.

NIH PM 3040-2, Section F13.B.15. (ACUC RESPONSIBILITIES) requires that the ACUC oversees the NIA's training program and ensures the program's effectiveness by:

- Tracking all PI and animal users' completion of mandatory training (see below) and verifying with PIs that the animal users are experienced or have received training on technical, hands-on procedures they will be responsible for conducting.
- Verifying that all animal care staff are experienced or have received training on technical, hands-on procedures they will be responsible for performing.
- Identifying training needs for intramural staff who work with laboratory animals, though it is often logistically unfeasible to send NIA staff to NIH main campus for such training.
- Advising the SD regarding the requirements for training of professional and technical staff in animal care and use.

REQUIRED TRAINING:

NIH-MANDATED:

1) ***PIs****: NIH policy (PM 3040-2) Section F.4.b.* requires each intramural PI using animals to complete a training course entitled "Using Animals in Intramural Research: Guidelines for Principal Investigators", before the investigator's ASP can be approved by the ACUC. The course consists of the following topics: laws, regulations, and policies; ethics and scientific issues; alternatives; responsibilities of the institution, ACUC, scientist, and veterinarian; ASP preparation and protocol management; pain and distress; anesthetics, tranquilizers, and sedatives; surgery and post-surgical care; euthanasia; and animal care and use. A refresher training course is required triennially.

2) ***Other animal users:*** *The Manual (Section F.5.a.)* also mandates that other researchers and technicians listed on the ASP complete a similar course, "Using Animals in Intramural Research: Guidelines for Animal Users," available as a web-based training module. Topics covered in this course are similar to those presented in the course for PIs, but with an emphasis on the proper handling, housing and care of research animals. The web-based courses contain hypertext links to web pages

containing the reference materials. A refresher training course is required triennially.

3) ***NHP users:*** "Working Safely with Nonhuman Primates"(lecture course). The NIH PM 3040-2 and PM 3044-2 both require all individuals regularly entering nonhuman primate (NHP) rooms to be trained in the safe handling of NHPs. The OACU Associate Director for Training, Education and Program Liaison, conducts a 'train the trainer' course for facility veterinarians, managers, or other key IC trainers, providing them information for conducting this NIH IRP training at individual work sites. Some of the CRL staff go to Redacted by agreemen periodically to observe/participate in routine NHP clinical procedures.

4) ***ACUC members***: "Animal Care and Use Committee Member Training: Defining the Challenge of ACUC Membership": provided by OACU. Course registration can be completed on the OACU training website: http://oacu.od.nih.gov/training/index.htm.

NIA/IRP-MANDATED (*Required for all PIs and animal users*):

1) CMS Animal Users Orientation: covers basic CMS policies and procedures for working with laboratory animals at the NIA, as well provides a walk-through orientation of our specific animal facility.

2) Basic Animal Handling Course: cover basics of rodent handling.

The two courses above may be combined in one training session.

a) Briefly describe the content of any required training.

Using Animals in Intramural Research: Guidelines for Principal Investigators (web-based training module). The course consists of the following topics: laws, regulations, and policies; ethics and scientific issues relating to research animal use, to include the 3Rs; alternatives; responsibilities of the institution, ACUC, scientist and veterinarian; methods for reporting concerns about animal care or use, ASP preparation and protocol management; pain and distress; anesthetics, tranquilizers, and sedatives; surgery and post-surgical care; euthanasia; and other topics relating to animal care and use.

Using Animals in Intramural Research: Guidelines for Animal Users (web-based training module). Topics covered in this course are

similar to those presented in the course for PIs, but with an emphasis on the proper handling, housing and care of research animals.

Animal Use Refresher Course for Principal Investigators & Animal Users (web-based training module). This course covers all the topics of the initial course, but in an abbreviated fashion. All active PIs and animal users must complete this refresher training every three years.

Working Safely with Nonhuman Primates (lecture course). The NIA ACUC Program Coordinator is the designated trainer for the NIA. The course includes viewing a video; "Working Safely with Nonhuman Primates," background information on occupational health and safety issues, and a quiz.

Animal Care and Use Committee Member Training: Defining the Challenge of ACUC Membership (lecture course). This two-hour interactive lecture course provides an overview of federal and local policies and regulations that govern animal care and use for the NIH intramural research program, and the ACUC's responsibilities for helping to meet the requirements.

CMS Orientation and Basic Animal Handling Course (lecture and hands-on course). Topics include institute policies, facility procedures, personal protective equipment, animal welfare, relevant contact information, rodent handling/restraint, aseptic microisolator technique, euthanasia, and a facility tour.

Non-animal users who may be operating within the animal facility:

George S. Hall (GSH) personnel may be required to operate within rooms containing animals. GSH personnel, who are dedicated, in-house maintenance management staff that may operate within the Redacted by agreement animal areas, go through all CMS orientation training. We ensure they are aware of the risks and protections required. Note that these individuals are also required to obtain occupational health clearance through their respective contracting companies, equivalent to NIH standards, if they will be operating in an animal area or potentially exposed to animal-related hazards (allergens, etc).

The ORF (Office of Research Facilities) coordinates any outside vendor visits, to ensure that any vendors coming in are properly cleared for entrance, appropriately escorted, or in other ways

protected. These personnel typically do not ever operate within rooms housing animals.

OTHER TRAINING (as-requested) and RESOURCES:

The OACU offers a hands-on mouse and rat workshop multiple times per year in Bethesda, Maryland. This course addresses handling and restraint, injection techniques, blood collection techniques and CO_2 euthanasia. This course is augmented by instructional video clips, currently posted on the OACU website, which are also used for general outreach and training purposes.

The CMS offers hands-on workshops on an as-requested basis to provide basic handling, restraint, and procedure training.

CMS financially supports sending investigative and CRL technical staff to mouse microsurgery training conducted by the National Heart Lung and Blood Institute (NHLBI).

Individualized training is offered as requested. Additionally, the ACUC coordinator and veterinary staff have interactive computer programs, videos, and an array of animal science books available to the investigative staff.

The CMS also maintains an intranet website for easy access to standard operating procedures, guidance documents, and training course registration.

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

Mandatory training must be completed before commencing work. As a check on this, no individual may be added to our approved protocol personnel database (required before protocol approval) without having submitted appropriate documentation to the ACUC.

c) Describe continuing education opportunities offered.

All NIA animal users have access to internal NIH/OLAW/OACU training opportunities, in person or via free webinar. Being part of the NIH community provides unique access to these opportunities, which are regularly and clearly publicized via internal NIH email. CMS also purchases webinars from AALAS on animal care and use-related topics. We have a relationship with National Institute on Drug Abuse (NIDA) that allows reciprocal sharing of such webinars.

All contract staff can attend one local conference annually and are allowed to attend national meetings if they present a topic as a poster or seminar.

Additionally, the ACUC coordinator and veterinary staff have interactive computer programs, videos, and an array of animal science books available to the investigative staff.

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]

Only rodent surgery is currently performed at the NIA; one rabbit subcutaneous telemetry implantation procedure, lasting less than 10 minutes, may be starting prior to our AAALAC site visit timeframe but it is not currently active as of submission of this PD (1 April).

Whenever surgery is planned for an ASP, documentation of surgical training and experience is also required on each ASP. This documentation is reviewed by the ACUC prior to ASP approval. Any questions regarding appropriateness of training is directed to the AV, who will determine by document review training records, previous protocols on which the individual has worked, etc.), discussion with the individual, or observation, that the individual is competent in the tasks to be performed.

Students are sent to other National Heart, Lung and Blood Institute NHLBI) for surgical training and may also be given protocol-specific training by in-lab trainers, as applicable.

When there are questions or concerns, the AV or her designee may review records, observe surgeries, or otherwise ensure that surgical procedures are performed in accordance with NIH Animal Research Advisory Committee (ARAC) Guidelines.

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

Only rodent surgery is currently performed at the NIA; one rabbit subcutaneous telemetry implantation procedure, lasting less than 10 minutes, may be starting prior to our AAALAC site visit timeframe, but it not currently active as of submission of this PD (1 April).

When applicable, documentation of anesthetic training and experience is required on each ASP and reviewed/approved by the ACU prior to ASP approval. Any questions regarding appropriateness of training is directed to the AV, who will determine by document review training records, previous protocols on which the individual has worked, etc.), discussion with the individual, or observation, that the individual is competent in the tasks to be performed.

Training is provided when needed. On behalf of the ACUC, AV or her designee ensures anesthesia procedures are performed in accordance with NIH ARAC Guidelines.

NIA SOPs provide detailed information on injectable and inhalation anesthesia and proper monitoring. Laminated instructional signage is posted near each machine regarding operation and safeguards.

PIs are responsible for training individuals in the specific anesthetic techniques used in their laboratories.

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

Methods of euthanasia are described in each ASP and all methods, to include any conditions for required for conditionally-accepted methods, are evaluated by the ACUC during the ASP review process.

Rodent euthanasia training using carbon dioxide is provided by CMS to all new researchers as part of mandatory, initial hands-on training. Training on other euthanasia techniques is provided by the CMS staff upon request. Principal Investigators are responsible for ensuring their research staff are proficient in performing all ASP-approved euthanasia methods.

All animal care and veterinary staff members receive hands-on instruction in carbon dioxide euthanasia and in performing a secondary physical method of euthanasia in rodents.
Detailed instructions on euthanasia are also posted at each carbon dioxide station.

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 occupational health and safety program incorporates the recommendations in the National Academies Press publications: *Occupational Health and Safety in the Care and Use of Research Animals; Occupational Health and Safety in the Care and Use of Nonhuman Primates; Guide for the Care and Use of Laboratory Animals*: Eighth Edition; as well as the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories (BMBL*): Fifth Edition.

The Office of Research Services (ORS) is the NIH management organization that establishes and implements policy and allocates resources required to support NIH's research mission and programmatic goals.

The Division of Occupational Health and Safety (DOHS) is the component responsible for developing, managing, and overseeing the occupational health and safety program (OHSP) and ensuring its compliance with NIH policy and applicable Federal, state, and local laws.

The ORS, DOHS, Occupational Medical Service (OMS) offers a full range of work-related medical services. The OMS designs and implements the Animal Exposure Program (AEP) for NIH personnel who are in direct contact with, are involved in the direct care of live research animals, or have contact with un-fixed, Old World NHP animal tissues or body fluids. The program is mandatory for these individuals, as specified in the NIH Policy Manual 3040-2.

Certified Industrial Hygienists (IH) are available as needed via DOHS.

The Division of Radiation Safety (DRS) specializes in radiation safety, regulatory compliance and risk management. They are available to provide technical radiation safety services and advice to the ACUC when applicable.

The Institutional Biosafety Committee (IBC) provides recommendations to the Director of the NIH in matters pertaining to the control of hazards associated with the use of microbiological agents, their vectors, and recombinant DNA. They are available to review and approve submitted documents for work with registered materials and to provide advisory support by recommending safety policies and practices.

The NIA/IRP has onsite staff to coordinate occupational medical services, emergency services, and to assist with establishing safety-related practices and procedures.

The NIA/IRP Safety Office consists of a Safety Manager, a Chemical Hygienist, and a Safety Specialist. This department provides occupational safety advice, guidance, and oversight to the laboratory animal research program. The office also provides mandatory safety training courses for new hires and a mandatory safety annual refresher course.

The Redacted by agreement is a member of the NIA ACUC and participates fully in the ASP review process. Her primary focus is to facilitate safety and hazard analysis communication and to ensure ASPs clearly identify the potential hazards through additional consultation with other NIH safety departments [i.e., ORS/DRS, ORS/DOHS/OMS, ORS/IBC, ORS/DOHS/Technical Service Branch (Laser Safety, Environmental Monitoring)].

An OMS nurse is located on-site for occupational health risk assessment reviews and is the medical professional who signs animal user occupational health clearances. She also provides emergency care for bites, scratches, exposures to hazardous substances, and other occupational injuries.

Contractors are required to provide an OHSP to their employees with animal contact that must be, at a minimum, equivalent to the NIH program. The CRL OHSP is monitored by the Animal Program Director. The contractors work closely with NIA personnel to ensure compliance with NIH standards and report injuries and potential hazards or exposures to the NIA Safety Office. While CRL staff (and their sub-contractors) may utilize onsite NIH OMS Services for acute work-related injuries, their routine OMS Services are provided by Concentra Urgent Care (closest location: 1833 Portal St; Baltimore, MD 21224).

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.].

The NIA Safety Office identifies, assesses, and manages risks at personalized and research planning levels. The office works closely with DOHS on NIA ACUC safety matters.

NIA Supervisors complete a "NIA/IRP New Hire Information Sheet" during the hiring process. The NIA Safety Office receives a copy of the sheets that indicate a new hire will have animal contact. The Safety Office contacts the new hire and refers FTEs (to include students and Fellow) to onsite OMS for medical risk assessment; CRL and other contract staff (housekeeping, security, maintenance) are evaluated by their employers' OMS. When needed, staff are medically cleared by OMS to use N95 Respirators and are trained and fit tested by the Safety Office to wear.

The NIH Animal Study Proposal (ASP) form has sections dedicated to: the identification of hazardous agents which may pose a potential hazard to other research, the animal care staff, or to the environment; and a description of the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with the study.

- Principal Investigators identify locations for performing animal procedures and holding in Section B, identify hazardous agents in Section K, and describe special husbandry practices and equipment in Section M of the ASP form. The ASP form also requires an NIH Human Pathogen Registration Document number for recombinant DNA or potential human pathogens used in the study.
- The NIH Safety Officer, as a member of the ACUC, contributes specialist input to ACUC review to ensure safe practices.

The NIH Safety Officer also coordinates review of the ASP and ancillary registration documents or appended safety instructions by the appropriate safety committees (i.e. DRS or NIH IBC). The NIA Safety Officer works with Principal Investigators and the CMS to develop and provide appropriate personnel training, engineering controls, protective equipment, and work practices,

The CMS has designated rooms for ABSL2 work. The NIA Safety Officer works with the CMS to verify that necessary safeguards have been incorporated into study procedures through routine survey of proposed work areas and assurance of appropriate signage.

The NIH and NIA Safety Officers participate in semiannual ACUC inspections and annual surveys of registered procedure rooms listed at ABSL 1, 2, and 2/3.

Information on animal bites, animal allergens, and zoonoses is provided through NIA Safety and the OMS (or contractor equivalent) Animal Exposure Program (AEP).

NIA follows the NIH Policy on "Medical Clearance of Transient Visitors into NIH Animal Facilities" [proponent is Director, Division of Occupational Health and Safety (DOHS), ORS]:

A transient visitor is defined as anyone who is required to enter an animal room, but not have direct contact with animals. Transient visitors include, but are not restricted to, federal employees and contractors, maintenance and construction workers, and members of the general public.

- All transient visitors to NIH animal facilities must be accompanied and supervised by an NIH employee trained in the safe entry into animal rooms.

- Transient visitors who enter an occupied animal room are required to wear appropriate personal protective equipment (PPE) as posted on the animal room door. Appropriate PPE for entry into animal rooms where infectious agents may be in use, must meet the requirements specified in the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories*. Transient visitors to NIH non-human primate (NHP) animal facilities must wear a single-use dust-mist mask and other PPE, as outlined in the NIH Policy Manual Chapter 3044-2, *Protection of NIH Personnel Who Work with Nonhuman Primates*.

- Prior to the entry of transient visitors into animal rooms to conduct significant facility construction, renovation or maintenance: 1) the animals must be removed from the immediate area; 2) the area must be cleaned and disinfected; and 3) the visiting personnel must be informed of any potential hazards and appropriate protective procedures, including required PPE.

The decision on whether medical testing is required for transient visitor entry into a NHP animal room rests with the facility Animal Program Director; **typically, they will be required to fulfill all occupational health requirements of animal users, but each case is evaluated individually.**

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

Hazards are assessed initially, prior to conduct of any animal work. Personnel are required to reassess their work requirements annually, and submit another assessment form when any changes occur, and receive medical clearance to work with animals again prior to continuing work.

The Redacted by Safety Committee conducts surveys of all areas annually, including procedure areas. The committee reports to the Scientific Director and is responsible for follow up.

The NIH Safety Officer conducts safety review of CMS as part of the ACUC Semi-annual facilities inspection, and the ACUC is responsible for follow up as part of the semi-annual review process.

Precautions for the use of hazardous agents in laboratory animals are fully documented in each ASP. The NIH Safety Officer (who represents the NIH Division of Occupational Health and Safety) reviews all ASPs for appropriate safety procedures for hazardous substances and is available to verify training or implementation of those procedures.

There are 2 CMS representatives on the NIA Safety Committee.

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]

As part of their initial and periodic health and safety training, employees are instructed to report observed hazards, exposure incidents, work-related injuries and illness to their immediate supervisor and to the NIA Safety Office. Injuries are reported to OMS and/or the NIA Safety Office.

As injuries are reported to OMS, they are entered into an electronic database for DOHS investigation and follow-up. The database allows

for specific metrices to be tracked: type of injury, location of injury, frequency by group/individual, and time to close incident.

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.

NIA Supervisors complete a "NIA/IRP New Hire Information Sheet" during the hiring process. The NIA Safety Office receives a copy of the sheets that indicate a new hire will have animal contact. All FTE's noted as working with animals must complete onsite OMS medical risk assessment. Staff that are noted as working with animal tissue only may not be required to complete OMS assessment.

b) Describe provisions for allowing an individual (following completion of individual health and job-related risk assessments) to decline participation in all or part(s) of subsequently available medical and preventive medicine components of the institutional program, e.g., vaccinations, physical examinations, respiratory protection, as applicable. Provide an estimate (percentage) of personnel associated with the animal care and use program that have declined participation in the medical evaluation program.
Note: Do not include names of the personnel

As noted above, all FTE's noted as working with animals must complete onsite OMS medical risk assessment. Based on job function and medical assessment, staff may be offered additional preventive medicine components e.g., vaccinations, respiratory protection, as applicable. And while staff can decline, staff are strongly encouraged to receive preventive components (or alternative when applicable) based on the associated risks. Currently

there are no NIA staff that have declined additional medical components.

c) Describe provisions for assuring confidentiality of medical information.

All federal HIPAA requirements are met when handling or using medical information provided as part of the occupational health/preventive medicine program. All OMS medical records are kept locked in the OMS Suite within a locked file cabinet. Only OMS staff have access to the Nurse's Office; only those qualified medical professionals with a need to know have access to these records. Medical clearance forms provided to NIA Safety or other personnel do not contain any HIPAA-protected information.

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

There are animal use signs throughout the building where animals may be present; these signs warn of potential allergens. There are also personal protective equipment signs that instruct individuals what is required to protect themselves upon entering a dedicated animal use space.

Training: all maintenance or other non-animal-users that might enter animal holding space are required to take the initial orientation provided by CMS. They also must obtain medical clearance for working in these areas. Outside vendors do not have to meet these two requirements, however, they are escorted while in the animal facility and are made aware of any potential hazards prior to entering the facility.

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.

All FTE's working with animals (as new hire or additional duty assignment) must complete onsite OMS medical risk assessment based on the animal type (rodents, NHP). The assessment includes

general health questionnaire, immunizations, animal related hazards (zoonoses, allergy prevention, and personal protective equipment). Staff are encouraged to return to OMS if health related issues arise. Staff working with NHPs are recalled annually for PPD testing.

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.

In addition to routine OMS services, the following provide emergency care, after-hours care, special medical evaluations as needed for all NIA associated staff (including contractors): NIH OMS Bethesda, Concentra, Patient First and Johns Hopkins Bayview Emergency Department. Each facility is familiar with NIH's AEP because they are currently supporting NIH staff or have in the recent past.

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.

NIA staff having animal contact receives training concerning zoonoses, personal hygiene and occupational health and safety through the Division of Occupational Health and Safety (DOHS). The DOHS sponsors courses in Laboratory Safety, and Retrovirus and Blood Borne

Pathogen Safety. The IRP Animal Exposure Program (AEP) provides educational materials concerning allergy prevention and zoonoses.

The CMS staff receives instruction per standard operating procedures and individual counseling, as necessary, from their supervisor, the Facility Manager or the CRL Corporate Trainer. The contractor trains its employees with programs meeting NIH specifications. Contract staff also receives AALAS training courses.
Personnel working with hazardous agents are senior technicians that have worked in barrier conditions and are trained via SOPs in containment procedures.

Prior to starting a new project in an ABSL-2 suite, investigators and their staff meet with CMS personnel assigned to the suites. Investigators provide a layman level presentation about the project and staff are encouraged to ask questions. The Safety Officer often joins these sessions.

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.

Most animals within the facility are mice, followed by rats, with the occasional presence of guinea pigs and rabbits. PPE required for operation within animal facility hallways is shoe covers only. For work within animal rooms of any species, personnel must additionally wear a facemask, bonnet, disposable lab coat and gloves. After work within an animal room is complete, the lab coat is put in trash; if it is still clean and there is an available hook in the room, it may be rehung within the room.

Note that while we do not currently house NHPs (and when we do, they are Redacted by agreement per month) when they are present PPE requirements include a Tyvek suit instead of disposable lab coat and in all cases, eye protection and an additional layer of gloves.

b) Describe arrangements for laundering work clothing.

Animal care and veterinary technical staff (i.e., those who perform recurring and/or extended duties within the animal facility) have

dedicated scrubs and work shoes that do not leave the animal facility. Clean scrubs and work shoes are kept in the locker rooms and donned at the beginning of the work day. At the end of the work day, or if departing the animal facility for any reason, the scrubs are placed into a wash bin and shoes are stored back in personal lockers. Personnel with dedicated shoes do not need to wear shoe covers while in animal corridors but must spray their shoes prior to entry and exit of the animal corridors. There is a washing machine and dryer on-site for laundering scrubs.

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.

Individuals are expected to wash hands after handling animals within animal rooms. Observing animals only does not require hand-washing. Sinks are located within or close to animal rooms (adjacent labs).

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

As per the Biosafety in Microbiological and Biomedical Laboratories (BMBL) and our internal policies, there is no smoking, eating or drinking (among other restricted activities) within the animal housing area of the animal facility (hallways, animal housing rooms and procedures rooms past the double doors).

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 only areas that potentially reach a time-weighted average of 85 dB or greater are the cagewash areas, for which hearing protection in the form of earplugs or earmuffs is provided. Relatively large quantities of washing chemicals are stored in a room adjacent to the loading dock; in-house staff does not handle or manipulate these chemicals (all such activities are handled by the vendor, who brings their own PPE as required).

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

The most likely source of allergens is the urine produced by mice, rats, other rodents, and, rabbits; staff can be exposed to these allergens through animal handling, cage changing, bedding dumping, and dirty-side cagewash processes, among others. A variety of measures are used to protect personnel from development of laboratory animal allergens. Individual PPE helps prevent hand-to-mouth and general skin contact exposure. Individually-ventilated caging systems, which house most or all our animals, depending on the timeframe, significantly reduces the level of allergens escaping into the atmosphere. Bedding dump stations are employed in dirty-side cagewash. Biological safety cabinets are used within animal rooms for most animal out-of-cage manipulations, to protect the individual and the environment from animal allergens.

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

The most likely source of zoonoses is through animal bites or scratches. Personnel are trained prior to any work with animals (during their initial orientation training and animal handling training) regarding how to respond to bites or scratches in order to minimize their risk of contracting zoonoses. Animal handling training is another method used to minimize bites and scratches, since an increased level of comfort in animal handling results in decreased stress to both the animal and the human involved, making bites and scratches less likely.

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

Individual PPE is all disposable, and as such, no maintenance occurs.

e) Respiratory Protection

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

Surgical-type or dust/mist face masks are provided at PPE dispensing stations at the entrances to the vivarium for voluntary-use users, and at cage wash areas. NIA SOPs requires all dust/mist masks use by cage wash personnel when scraping dirty bedding from rodent cages. While these are not considered "respiratory protection" they may provide some protection from certain particles carried on mist droplets.

True respiratory protective equipment, including respirator masks conforming to the NIOSH N95 standard, may be required for individuals participating in animal study proposals (ASP) that involve hazards and for individuals with documented allergies to laboratory animals.

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

Occupational Medical Services (OMS) provides medical evaluation and clearance to NIH employees.

The DOHS Respiratory Protection Program is locally implemented by the NIA Safety Office.

- Mandatory and voluntary-use users of class N-95 respirators are required to participate in the program.
- The program provides worksite evaluations, respiratory selection guidance, respirator training and fit testing with annual recall. Appropriate respirators are provided to employees by the NIH at no cost to the employee.

Contract animal husbandry and research staff receive medical clearance and fit testing from their employer's occupational health and safety providers.

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

N95 respirators are worn by individuals with documented allergies to laboratory animals. The Safety Office maintains a variety of manufacturers, models and sizes for fit testing via the Port-a-count method. Once properly fitted, staff are recalled annually to re-test.

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).

The NIA animal facility uses 1 cage/rack washer, 2 tunnel washers, and 3 bulk autoclaves; all are in the walls between clean and dirty side cagewash except for the 2 autoclaves on clean side and one on dirty-side cagewash.

New hires go through a three-step process: read SOPs, perform the duty under supervision, then are signed off to work independently when competent to do so. Care is taken to emphasize safety precautions, especially with regards to our walk-in equipment, where emergency egress may be required.

GSH also provides individual operator basic safety and operator maintenance for everyone, and we often have the vendor participating in these discussions to ensure we learn of any changes in procedures or best practices from the manufacturer.

Signage: Signs are located outside each cage-rack washer and tunnel washer regarding emergency shut-off procedures; signs are located near the actual buttons to be pushed in case of emergency. Cage/rack washers also have emergency pull cords inside.

For all bulk autoclaves, ON/OFF operation is controlled by an external key. When the key is removed the autoclave will not operate. This key must be removed and *worn in a visible location* by the loader prior to commencing any work within the autoclave. It is replaced after loading is complete and personnel are outside the autoclave.

Forklift: We have one motorized, non-riding forklift. Training is provided to all operators by the Animal Resources Program Manager.

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.

N/A

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.

Animals are only rarely transported from the NIA to other locations using NIA vehicles. Animals are transported in clean, filter top containers that will minimize allergen dispersion within the vehicle. Drivers are trained on the hazards of animal work.

There are no instances of live animals and personnel transport at the same time.

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

F/air canisters or active scavenging systems are the methods by which volatile anesthetics are scavenged.

Active scavenging is accomplished by snorkels directly vented through house WAG lines. No stand-alone active systems are used.

Waste anesthetic gases are quantitatively monitored periodically by DOHS personnel, or when requested by laboratory or CMS staff. Quantitative measurement (surveys) are needed on a recurring basis; quantitative measurements are also required for new studies that use new types of anesthetic setups. Quantitative measurement is accomplished by Industrial Hygiene (IH) personnel from DOHS.

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.

Biological Agents	Toxin	BSL1	BSL2
4T1 breast cancer cells		x	
Adenovirus		x	x
Anti-mouse CD4 GK 1.5		x	
B16F10 melanoma cells			x
Complete Freunds Adjuvant (CFA)	x		
Diptheria	x		x
DNA-constructs		x	
Extracellular Vesicles		x	
Glucagon-like peptide-1 (GLP-1) Agonist		x	
Glycoprotein deleted rabies virus			x
Human exosomes			x
Human fecal suspension			x
Intestimonas butyriciproducens		x	
Lentivirus		x	x
Lipopolysaccharide (LPS)	x		
Listeria monocytogenes			x
Mesenchimal Stem Cells			x
Micro-RNA		x	
Pertussis Toxin	x		x
Picrotoxin	x		x
Preformed alpha-synuclein fibrils	x		
Rabies			x
Rat amniotic epithelial stem cells		x	
Replication incompetent retrovirus		x	x

sRAGE		x	

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

Chemical Agent	AKA	Toxic/Toxin	Irritant	Carcinogen	Mutagen
2,4-Dinitrophenol	MP-101; DNP	x			
2-Methylbutyric acid	2-MBA	x			
3-Nitropropionic Acid		x	x		
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) solution	NNK	x	x	x	
5-Bromo-2′-deoxyuridine	BRDU	x			x
5-ethynyl-2'-deoxyuridin	EDU	x			x
5-flurouracil	5-FU	x		x	
6-hydroxydopamine	6-OHDA	x	x		
Acrylamide		x	x	x	x
AICAR		x	x		
Atropine		x			
Azoxymethane		x	x	x	
Benzo[a]pyrene		x	x	x	x
Carbachol		x			
Carmustine	BCNU	x		x	x
Chlordiazepoxide hydrochloride		x			x
clozapine-N-oxide	CNO	x	x		
Colchicine					
Collagenase		x	x		
Concanavalin A	Con A	x	x		
Cyclophosphamide		x		x	

Desipramine		x	x		
Dextran sulfate sodium salt	DSS	x			
Diethylnitrosamine	DEN	x		x	
Dimethylbenz[a]anthracene	DMBA	x		x	
Dioxin TPDD	TPDD	x	x		
DOI		x	x		
Formalin		x	x	x	x
Freunds Adjuvant Complete	CFA	x	x	x	
Gluteraldehyde		x	x		
Hydroxytamoxifen	4-OHT	x			
Indomethacin		x			
Isoflurane		x			
Isoniazid	INH	x			
Isotretinoin	13- cis-Vitamin A acid	x			
Kainic Acid		x	x		
Kynurenine		N/A			
Lenalidomide		x			
Lipopolysaccharides	LPS	x			
Mepivicaine		x			
Olaparib		x			
Paraformaldehyde		x	x	x	
Pentylentetrazole (PTZ)	PTZ	x	x		
Pilocarpine hydrochloride		x			
Pomalidomide		x			
Potassium bromate	KBrO3	x		x	
Propranolol		x			
Rotenone		x	x		

Sodium Pentobarbital		x			
Streptozotocin	STZ	x	x	x	x
Suberoylanilide hydroxamic acid	Vorinostat (SAHA, MK0683)	x			x
Tamoxifen		x		x	
Thalidomide Analogs		x			
TPA	PMA	x		x	

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

1) A Magnetic Resonance Imaging (MRI) suite is currently operational for rodents.

2) The only study currently using radioactive hazards is 325-TGB-2022:

   In this study 10 µCi 2-deoxy-d [1-14C]-glucose is used to measure glucose metabolism in different tissues. It is used as part of the HE-CLAMP procedure. The HE-CLAMP procedure is conducted by restraining an awake animal in a device that allows for repeated blood measurements of glucose turnover; total time is approximately 140 minutes, after which animals are typically euthanized.

   After 90 minutes from the start of the HE-clamp, blood glucose reaches steady state and at this time 10 µCi 2-deoxy-d [1-14C]-glucose is injected via the catheter as a bolus administration. This allows for measurement of the insulin-stimulated glucose turnover in the different insulin sensitive organs like skeletal muscle and adipose tissue.

   Procedures involving the use of radioactive materials will be performed in [Redacted by greement] a posted room approved for both animal use and radioactivity use. *Standard animal PPE is sufficient to protect users from exposure/effects of this agent.* Radioactive waste is removed according to established Division of Radioactivity Protocols by an approved waste service.

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.

The NIH Animal Study Proposal (ASP) form has sections dedicated to: the identification of hazardous agents that may pose a potential hazard to other research, the animal care staff, or to the environment; and a description of the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with the study.

- Principal Investigators identify locations for performing animal procedures and holding in Section B, identify hazardous agents in Section K, and describe special husbandry practices and equipment in Section M of the ASP form. The ASP form also requires an NIH Human Pathogen Registration Document number for recombinant DNA or potential human pathogens used in the study.
- The NIH Safety Officer, as a member of the ACUC, contributes specialist input to ACUC review to ensure safe practices.

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.

CMS SOPs or ACUC Appendices address technical procedures to be used to minimize risks with these items. CMS SOPs are reviewed and approved by a committee comprised of the Animal Facility Manager, Assistant Facility Manager, Regulatory Veterinarian, and other CMS veterinarians; any SOPs requiring additional biosafety expertise is also reviewed by our Safety Office; irradiator use SOPs are reviewed by the NIH Division of Radiation Safety (DRS). Appendices are reviewed and approved by the ACUC.

The NIH Safety Officer coordinates review of the ASP and ancillary registration documents or appended safety instructions by the appropriate safety committees (i.e. DRS or NIH IBC). The NIA Safety Officer works with Principal Investigators and the CMS to develop and provide appropriate personnel training, engineering controls, protective equipment, and work practices,

Any specifically occupational health-related or other questions for which additional expertise is needed are referred to the NIH Division of Occupational Health and Safety (DOHS).

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.

Chemical Waste: Chemical wastes, e.g. formalin solutions, cytotoxic agents, non-controlled drugs, empty solvent bottles, chemically contaminated gloves, are segregated by compatibility, accumulated, and managed in waste containers, identified with the appropriate fields completed and placed in secondary containment. Waste containers range from empty chemical bottles (labeled "Waste" or tagged with chemical waste tag after striking through the original bottle label) to carboys for larger (> 2 gallon) volumes of liquid wastes and sealed, medium weight, clear plastic bags for dry waste. Chemical waste is stored in the designated chemical waste storage room Redacted by reement or at the point of waste generation until ready for pickup. NIH policy requires waste storage not to exceed 60 days; however, requests for waste removal from laboratories is provided within 48 hours of the request to the chemical safety technician, and, routinely, twice a week. Removal of wastes accumulated in the buildings' chemical waste storage room is performed monthly.

Biological/Pathological Waste: Biological/Pathological waste, e.g. animal carcasses, tissues, sharps containers (scalpels, Pasteur pipettes, pipette tips, all needles, syringes) is managed at the source point. All Biological/Pathological waste is identified as such. Liquid MPW is disposed of in a compatible, waste container, which is labeled as multi-hazardous waste by addition of a chemical waste tag. Solid MPW, which includes closed sharps containers, is placed into a MPW box, which contains double red bags. MPW boxes are labeled to identify the facility and sealed with tape once ready for storage. The NIH Safety Officer prescribes procedures in the event MPW must be neutralized or converted to general waste through decontamination/inactivation before removal from the facility. Scheduled preventative maintenance is provided to autoclaves by contract. As above, MPW containers are removed from the laboratory point of origin, accumulated in a designated cold storage area, and removed twice weekly by a commercial MPW handler for incineration. Weight limit is 40 lbs maximum per box.

Radioactive Waste: any waste (including chemical and biological) that has come in contact with radioactive material must be discarded as

radioactive waste. Radioactive waste is collected in designated containers.

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

The NIA Safety Office provides technical support for monitoring and ensuring compliance with NIH safety policies. Potential exposure is reported to the area supervisor and the NIA safety office.

- All personnel must be trained by the PI in agent specific procedures before beginning work.
- Areas and cages which contain potentially hazardous materials are labeled with appropriate cautionary information.
- Proper PPE must be worn at all times while working with the agent.
- If an agent warrants being registered with the IBC, all personnel working with the agent must be included on the registration and be up to date with NIH safety training and any occupational health requirements, such as vaccinations. A chemical fume hood or biosafety cabinet is used to provide primary containment.
- Waste liquid hazards are held in labs within labeled safety cans or designated plastic or glass disposal containers for pickup by Division of Environmental Protection staff within 60 days of generation or less to minimize the amount of hazardous material.
- Potentially infectious materials are collected in biohazard waste boxes, sealed within two plastic bags, for disposal as medical waste.
- Substances such as paraformaldehyde and formalin are used in a chemical fume hood or downdraft table, and the personnel are required to wear the proper PPE. They are collected in a container and disposed of as chemical waste. Isoflurane is properly scavenged through both passive and active systems to minimize exposure.

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.

Personnel working with hazardous agents are senior technicians that have worked in barrier conditions and are trained via SOPs in containment procedures.

Prior to starting a new project in an ABSL-2 suite, investigators and their staff meet with CMS personnel assigned to the suites. Investigators provide a layman level presentation about the project and staff are encouraged to ask questions. The Safety Officer often joins these sessions.

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.

Animals exposed to hazardous biological agents are not housed within conventional animal rooms for the duration of risk to personnel.

Animals exposed to chemical hazards may be housed in conventional animal rooms.

The Redacted by agreeme has an ABSL-2 suite for mice exposed to hazardous biological agents. The mouse ABSL-2 suite consists of an animal holding room Redacted by agreement sq-ft) that will hold six racks and a procedure room Redacted by agreement sq-ft). ABSL-2 agents may be used in procedure room Redacted by agreement sq-ft), which is outside of an ABSL-2 suite. Cages are changed in a certified NuAire Class II, Type A2 biological safety cabinet.

Access to the ABSL-2 suite is controlled by an entry door proximity card reader. All rooms have a minimum ventilation rate of 10 air changes/hour; room pressure is negative to corridors. Exhaust air leaving the ABSL-2 area is filtered through a pre-filter and HEPA filter.

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.

Biosafety cabinets, downdraft tables, and chemical fume hoods are all engineering controls used at the NIA.

Animals exposed to biological hazardous agents are housed within dedicated animal rooms for the duration of risk to personnel. Animals exposed to chemical hazards are held in conventional housing. Rooms are posted with applicable hazard warning signs. Cages containing exposed animals are identified with cage cards watermarked with a chemical caution or biohazard symbol.

- At least one item of primary containment is required for all studies involving hazardous agents (e.g. Tamoxifen, BrdU, and lentiviral vectors) within animals. Primary containment may be one or more of the following items:
    - Static microisolator cage with filter media lid
    - Biological Safety Cabinets or laboratory fume hoods (for dosing and researcher provided husbandry of cages containing potentially hazardous wastes)
- Room ventilation balance is used for secondary containment, i.e. directionality is adjusted for air to flow into the animal holding/manipulation area and maintain a negative differential pressure to its room or adjacent corridors and rooms. Types include:
    - General and animal laboratories have negative differential pressure to corridors.

Chemical fume hoods (rooms Redacted by agreement and Redacted by agreement or downdraft tables (room Redacted by agreement are used when handling hazardous chemicals (e.g. Tamoxifen, BrdU, formalin).

Isoflurane anesthetic gases are scavenged through local exhaust ventilation (LEVs) systems to non-recirculating, building HVAC exhaust, waste anesthetic gas vacuum systems, and/or adsorption in canisters (e.g. f/AIR) to minimize personnel exposure.

A NuAire Portable Animal Bedding Disposal Cabinet is used for dumping cages containing bedding contaminated with chemicals designated as dump to burn.

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

Animal care personnel do not handle animals when they could potentially shed or release hazardous agents. Details of length of time animals may be hazardous are provided in the ASP and care staff will not handle animals until that time.

Special signage is put in place when hazardous materials are being handled in the BSL-2 rooms. During these times, animal care staff will not enter the room.

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.

There are no common animal/patient areas. For times when animals may be transported through human common-use areas, see section below.

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.

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, the methods and containment to be utilized must be described in the ASP (there is a section specifically for this).

There is one freight elevator that can transport items between the Redacted by reement floors. Animals or animal products may be transported in this elevator. Signage exists that notifies personnel regarding this potential use.

Infrequently, animals may be transported between the NIA and the National Institute for Drug Abuse (NIDA), co-located within the building, requiring transport of animals across common-use areas.

In this case, specific SOPs address this transport, to include measures taken to minimize any associated risks. These include placing animals into clean transport containers, covering the container, and using a clean cart or individually carrying the clean animal transport cage to the other handoff location. In no cases will someone who has been in the NIA rodent facility enter the NIDA rodent facility or vice versa.

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.

The Scientific Director (SD), through delegated authority from the Deputy Director for Intramural Research/Institutional Official (DDIR/IO), appoints the ACUC Chair, Vice Chair and members.

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

The NIA ACUC meets monthly to conduct regular business. In addition, emergency meetings may be called to discuss animal-welfare-related reported concerns or similarly urgent matters.

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

Following appointment to the committee, new members receive a copy of the Guide and attend the NIH Office of Animal Care and Use, "Animal Care and Use Committee Member Training: Defining the Challenge of ACUC Membership" course. This two-hour interactive lecture course provides an overview of federal and local policies and regulations that govern animal care and use for the NIH intramural research program, and the ACUC's responsibilities for helping to meet the requirements. After conclusion of this formal training, new members are briefed by the ACUC chair and/or the ACUC program coordinator with respect to routine and customary procedures employed by the committee as well as detailed instructions on the ASP review process.

The ACUC program coordinator maintains a library of resources available to the committee members and informs the committee of relevant continuing education opportunities (webinars, seminars, workshops, etc.). Certain ACUC members attend courses such as IACUC 101/etc., NCAB, and AALAS. All ACUC members are also invited to any animal care and use-related webinars provided to other staff.

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 ASPs are reviewed in light of the relative harm-benefit that may result, and all Committee members are aware of this ethical mandate. This is one reason why we currently review every protocol in full Committee, regardless of species used.

The expectation is that the ASP must present a clear description of the animal procedures. This standard is met through written text, simple flow charts or other diagrams, and deliberations and requested modifications of the ACUC during review. The ACUC's composition ensures a well-rounded, knowledgeable committee, and they collectively ensure the procedures are understood and animal welfare concerns are discussed and addressed.

As the potential impact of proposed procedures on animal well-being increases, the ACUC weighs procedures against potential animal welfare concerns; they consider the study description of alternatives considered, scientific justification of the approximate number of animals required, and experimental refinements. ASPs with procedures that have the potential for

more painful or distressful adverse effects typically garner more discussion by the ACUC.

For new experimental paradigms or pilot studies, applying statistical principles to justify numbers can be difficult due to the lack of knowledge regarding potential outcomes and variance. Under these circumstances, investigators are advised to mathematically or in some other relatively objective manner show how estimated animal numbers were approximated using the best information possible; they must assert that the sample size reflects the minimum number of animals required using the best information available.

Any protocol involving procedures that can cause pain or distress (USDA Column D or E animals) requires a literature search for alternatives to these painful and distressful procedures. PIs must consider these alternatives, if available, and if applicable justify why these alternative procedures cannot be used for their specific study.

If the ASP involves animals in USDA Pain Columns D or E, prior consultation with the AV is required to discuss pain and distress assessment and control, surgical preparation/anesthesia/technical procedures/postoperative care and euthanasia, although the veterinarian typically provides input on any other study aspects relating to animal procedures and welfare. Veterinary input is provided either before or during the pre-meeting review period (the period prior to ACUC meeting during which pre-reviewers may make comments). Input is provided by the Regulatory Veterinarian (who is also the ACUC AV), either in person, by phone, or by email.

Protocol approval process: All proposed animal research is described by the PI on the standard NIH Animal Study Proposal (ASP) form utilizing the web-based Internet Animal Study Proposal (IASP) system (example forms are provided in Appendix 7). Requests for changes to animal activities (amendments) are also submitted via this electronic system. Once the document has been submitted, it follows the following process:

- ASPs/amendments to existing ASPs are submitted to the PI's Laboratory/Branch Chief for review of scientific merit and signature of approval. Laboratory/Branch Chiefs submit their ASPs to the NIA SD for approval.

- Signed documents are then forwarded to the ACUC Coordinator. The ACUC coordinator provides a pre-review to ensure that all sections of the ASP are completed. If an ASP involves *in vivo* animal

intervention, PIs are encouraged to consult with the Regulatory Veterinarian prior to finalizing and submitting the ASP.

- After document submission, and prior to the ACUC meeting, the ACUC Coordinator assigns a primary and secondary reviewer to each submission. The AV is an additional reviewer for all documents submitted to the ACUC for approval.

- 2 weeks prior to the monthly ACUC meeting, the ACUC Coordinator distributes an agenda to the committee. All committee members have access to all submissions on the agenda.

- At the meeting, the full ACUC committee (Full Committee Review, or FCR) reviews and discusses each submission as presented by the assigned reviewers. The ACUC then formulates its disposition. For each submission the Committee votes to: 1) Approve; 2) Require modification to secure approval (with revisions reviewed/approved by Designated Member Review or full Committee re-review); or 3) withhold approval. Approved submissions are finalized by the signatures of the Attending Veterinarian and the ACUC Chair. Minority opinions are captured when they occur.

- The DMR process used subsequent to FCR will result in a possible outcome of: 1) Approved; 2) Requires modifications to secure approval; or 3) Returns to FCR.

- Although rarely utilized, submissions requiring action between regularly scheduled meetings may be provided an accelerated review using the DMR process. Copies of the submission are provided to each committee member along with assignment of a primary and secondary reviewer. Within five business days of distribution, each ACUC member is asked to provide their approval of the use of DMR or their request for a full committee review. If approved by the Committee, the DMR process is performed with the same possible outcomes as for its use subsequent to FCR: 1) Approved, 2) Requires modifications to secure approval, or 3) FCR. Any Committee member may call for FCR at any time during the DMR process.

- This accelerated review process using DMR from the start is utilized only under exceptional circumstances; PIs are strongly encouraged to use the routine ASP submission and review process.

- All approved documents are finalized by the signatures of the ACUC Chair and the Attending Veterinarian. Approved ASPs and meeting minutes are filed in the office of the ACUC Coordinator.

- ASPs are approved for a maximum of three years.

- Annual reviews: Once each calendar year, all approved ASPs are reviewed by the PI and an Annual Review form is submitted to the ACUC for approval. The Annual Review form is presented at a convened meeting for approval and subsequently signed by the ACUC Chair and Attending Veterinarian.

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.

According to the ACUC Policy, "Changes to Animal Study Proposals" amendments are reviewed by the following different methods, depending on the nature of the amendment:

Minor Amendments:
Minor changes *(i.e., non-significant changes to animal activities*) may be handled administratively by the ACUC Coordinator and do not require full committee review by the ACUC. These include:

- updates to contact information;
- typographical corrections; and
- personnel additions or deletions (the ACUC Coordinator reviews training documents for every new individual listed in an amendment to verify that they have adequate training documentation for the procedure and species to be used; she also reviews their non-HIPAA-controlled occupational health clearances to ensure they are allowed to work with animals from a medical standpoint. If any questions arise regarding training, she consults with the ACUC AV / Veterinarian for Regulatory and Policy Compliance; if occupational health questions arise, she contacts the ACUC's safety point of contact or occupational medical personnel located within the building.)

Major Amendments:

Significant changes to animal activities are considered major amendments, which may be handled in one of three ways:

1) Full Committee Review (FCR). Significant changes handled by this route include:

    - addition of a prolonged restraint procedure;
    - addition or change in use of hazardous agents or other changes that could affect personnel safety;
    - addition of animals in excess of 10% of the currently approved number or addition of animals in excess of 50% of the currently approved number for issues specifically related to mouse genotyping and breeding;
    - addition of any procedures or methods potentially resulting in an increase in pain or distress;
    - addition of other procedures not covered by a currently approved SOP or Appendix;
    - change from non-surgery to surgery;
    - change in PI;
    - change in species;
    - unconventional housing or husbandry requests that do not meet standards within the Guide; and
    - requests to house animals outside the vivarium for longer than 12 hours

Note that an accelerated review using the DMR process, described above for ASPs, may also be used in for these kinds of changes.

2) Veterinary Verification and Consultation (VVC). A subset of *significant changes to animal activities* may be administratively reviewed and verified by the AV. These changes include:

    - Changes in anesthesia, analgesia, or sedation that are considered equally effective and reliable; established CMS SOPs provide the most commonly used protocols. However, in consultation with the investigator, the AV may recommend changes in anesthesia and analgesia using doses from published formularies, recent literature, or generally-accepted veterinary medical procedures;
    - Changes in experimental substances similar to agents/articles in the currently approved ASP;
    - Change in method of euthanasia if an approved method in the AVMA Panel on Euthanasia;

- Change in duration, frequency, type, or number of procedures performed that have no effect or decrease level of discomfort/distress of the animal *and* are described in an approved CMS SOP or ACUC Appendix; and
- Increases in animal numbers up to but not exceeding 50% of the currently proposed amount for issues specifically related to mouse genotyping and breeding.

3) A final subset of changes can also be made administratively; these changes include:
   - Increases in animal numbers that are less than 10% of the currently approved amount (given that requested increases are not due to animal deaths or unexpected events); and
   - Addition of a strain, stock, or genotype with no anticipated adverse phenotype.

Amendment Review Process:

The ACUC Coordinator acts under the authority of the ACUC to evaluate the submitted change in light of the ACUC-approved policy on what constitutes each type of change to determine its category and, if significant, whether it must be reviewed by the full committee, VVC or the administrative route. When there is doubt, the AV and Chair will also consult to determine appropriate processing. (In some cases, the change may deviate enough from the original scientific aims, methods or species of the study that a new protocol is required).

The requested change, if significant and requiring full committee review, will be processed in the same manner as ASPs (they are discussed at the next meeting of the ACUC, provided appropriate deadlines for submission have been met).

As with ASPs, although rarely utilized, change submissions requiring action between regularly scheduled meetings may be provided an accelerated review using the DMR process. Copies of the submission are provided to each committee member along with assignment of a primary and secondary reviewer. Within five business days of distribution, each ACUC member is asked to provide their approval of the use of DMR or their request for a full committee review. If approved by the Committee, the DMR process is performed with the same possible outcomes as for its use subsequent to FCR: 1) Approved, 2) Requires modifications to secure approval, or 3) FCR. Any Committee member may call for FCR at any time during the DMR process.

All changes not requiring full Committee review are also included on the agenda for the next ACUC meeting to allow for complete transparency to Committee members for all activities occurring between meetings; this also allows for questions or additional discussion as required.

Once changes to animal activities are approved, other persons impacted by the changes are notified by email (this includes the Animal Facility Staff and other support staff).

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.

The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. Experimental endpoints relate to the project goals and are often either a timeline of data collection or desired clinical outcomes that provide proof or disproof of the experimental hypothesis. Humane endpoints are those chosen strictly to minimize pain and distress, and once reached will lead to animal removal from study (either through euthanasia or discontinuation of experimental manipulations).

In aged animals, experimental endpoints may overlap with humane endpoints because these studies often use the appearance of clinical signs as a signifier that a certain disease process has been successfully induced. It therefore may be difficult to differentiate between the two, and thus both endpoints (experimental and humane) must be kept in mind when assessing any animal exhibiting signs of pain or distress, lest valuable data be lost or, conversely, lest an animal be held on study longer than necessary.

All Animal Study Proposals (ASPs) must define both experimental and humane endpoints. PIs must also justify use of endpoints that occur after the onset of pain or distress (USDA Column E animals). At this Institute we acknowledge that, even if an animal will be euthanized reaching a certain point, in certain cases there may be a relatively unpredictable onset of certain age-related conditions and therefore cases where the age-related condition occurs after regular hours); in these cases every effort is made to predict as closely as possible the

number of animals that may be so affected, and categorize them into USDA Column E.

The NIH ARAC Guidelines for Endpoints in Animal Study Proposals recommend that experimental endpoints be carefully considered in order to minimize the pain and distress experienced by animals used in research, and that the earliest, most predictive indicators of clinical outcome be used. Optimally, the experimental endpoints should be before, or at the beginning of the period when animals exhibit clinical signs of disease or distress (i.e., morbidity). If compatible with achieving the research objectives, such endpoints are strongly preferred to significant morbidity, moribundity, or mortality (death) as endpoints. Scientific justification appropriate to the degree of pain or distress involved for the use of morbidity, moribundity, and mortality endpoints must be provided in the ASP submitted to the NIA Animal Care and Use Committee (ACUC).

In designing an experiment and considering humane endpoints, the scientist is encouraged to:

- Consider, at the planning stage, the experimental result they wish to achieve and what might be the earliest observable indicators of that effect prior to the animal experiencing pain/distress.
- Develop specific endpoints for each experiment, after consulting related literature, other scientists and the attending veterinarian.
- Balance the scientific output of the project with the minimum of adverse effects on the animals.
- Ensure appropriate training and competency for all those engaged in monitoring animals for signs of adverse effects and implementing humane endpoints.
- Investigators conducting studies on longevity and lifespan are advised to use endpoints suggestive of approaching mortality. Death as an endpoint is not encouraged. The ACUC has developed a policy specifically addressing aging studies to aid in the development of these endpoints.

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).

Investigators proposing studies for which little information is available on suitable endpoints are encouraged and often required to conduct pilot

studies in close consultation with a CMS veterinarian. The CMS veterinarian provides monitoring of animals throughout the pilot study period and aids investigators in determining suitable endpoints and in developing appropriate pain and distress monitoring systems.

Per the ARAC Guidelines, ASPs utilizing death or moribundity as an endpoint must contain the following information:

1. The scientific rationale for death or moribundity as an endpoint, including:

a. What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible. Why measures to relieve pain and/or distress cannot be utilized.
b. The number of animals that will be allowed to reach moribundity/death and justification for it being the minimum necessary to achieve the scientific objectives.
c. Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.

2. A plan for the following animal care and monitoring procedures:

a. Animals involved in experiments that may lead to moribundity or death will be monitored by animal care personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least the following: abnormal posture, rough hair coat, head tucked into abdomen, exudates around eyes and/ or nose, skin lesions, abnormal breathing, difficulty with ambulation, decreased food or water intake, or self-mutilation. As noted previously, monitoring by CMS staff occurs twice daily Monday to Friday during regular business hours (7:00 AM to 3:30 PM) and once daily on weekends and holidays.
b. The frequency of observation must be increased when animals exhibit the above or other signs of morbidity. Therefore, monitoring on evenings, weekends, and holidays will require involvement of the investigative staff to supplement observations by the animal care and veterinary staff. A system of increased observation should be established and in place for animals that exhibit deteriorating conditions that do not yet meet established ASP endpoints. Furthermore, the ACUC may prescribe more frequent monitoring for certain models.

c. Consideration will be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely. Dead animals must be promptly removed.
d. Written records must be kept of all monitoring and treatment.

The NIA ACUC **does not** recommend using death as an endpoint in aging studies. Indeed, there are changes in the clinical condition of an aged animal that may reliably predict impending death (mice: Whitehead et al. 2014, Trammel et al., 2012) and, when used, allow Column C classification (see NIA ACUC Policy, Classification of Animals in the USDA Reporting System).

The NIA ACUC therefore suggests that presence of any one of the following clinical signs be considered an endpoint in natural aging studies:

1. Loss of righting reflex
2. Prolonged anorexia (In NHPs, this is defined by a failure to consume enough calories to maintain a calculated resting metabolic rate. NHPs are tube fed for initial clinical support.)
3. Weight loss
    a. Rodents – Acute and unexpected weight loss that is greater than 20% from an individual established baseline weight. This weight is typically measured prior to the application of an experimental manipulation or is the maximum weight attained in adulthood (with an absence of any tumor burden).
    b. NHPs – 10% loss from the onset of a medical condition and in conjunction with adverse health conditions
4. Body Condition Score of 1.5/5 (mice: Ullman-Cullere & Foltz 1999; rat: Hickman & Swan 2010; macaque: Clingerman & Summers 2012)
5. Ear infection as evidenced by a head tilt, accompanied by spinning, and refractory to treatment (rodents)
6. Non-resectable tumors that meet established endpoint criteria (rodents; ARAC Guidelines for Pain & Distress)
7. Unresolved rectal/vaginal/uterine/penile prolapse
8. Firmly distended abdomen with evidence of respiratory distress (rodents)
9. Progressive body temperature declines over a period of weeks despite clinical intervention.
10. Clinical conditions (e.g. diarrhea, vomiting, dehydration) refractory to treatment (NHPs);
11. Blood work indicative of organ failure (NHPs)

12. Persistent signs of pain and/or distress: Signs of pain and distress vary by species and many animals may exhibit only subtle changes in behavior (we use NIH/ARAC Guidelines for Pain and Distress in Laboratory Animals as a main reference).

Further discernment of pain behaviors in any NIA species can be aided by consultation with a CMS veterinarian.

In addition to these signs, it is encouraged that investigators develop endpoints that suggest impending death based on experience with individual rodent models, strains, stocks, and genotypes; model-specific endpoints should be as objectively described as possible in the ASP.

It is also expected that aging animals may exhibit benign morbidities that would otherwise suggest pain and distress in a younger animal. One example is the kyphosis of the aging spine, which resembles the hunched posture seen with dehydration in a young mouse. Aging animals may also experience certain ailments at an increasing incidence; typical examples are skin conditions or cutaneous tumors.

Animal care staff are trained in expected conditions in aging animals which may necessitate treatment and veterinary consultation, include:

1. Abnormal posture/kyphosis
2. Unkempt hair coat
3. Increased discharge from eyes and/or nose
4. Skin lesions (e.g., necrosis, dermatitis)
5. Altered respiration
6. Slowing or atypical ambulation
7. Decreased food and/or water intake
8. Cutaneous tumors
9. Chronic weight loss

Treatment of any condition beyond basic supportive care and the application of topical medications should occur following discussion between the veterinarian and investigative staff.

NIA animal care staff follow the following specific procedures with regards to Column E animals; these procedures are detailed in CMS SOPs:

Column E status is assigned to animal(s) that are perceived to be experiencing scientifically justified, unrelieved pain and/ or distress that is explicitly approved by the Animal Care and Use Committee and

documented in the Animal Study Proposal (ASP). Expected clinical signs and subsequent management of animals approved for this status are described in Section F of the ASP and in the supplemental Column E Justification Form. Furthermore, the Instructions for Emergency Treatment and Care (IETC) form provides contact information for the lab if emergent questions or issues arise.

Research personnel are expected to inform the veterinary staff members of upcoming procedures or models categorized as Column E. Column E animals may initially be identified at a stage when distress/pain is unrelieved or may progress to that point during the course of clinical observation. Depending on the model and the approved ASP, cages may be either designated with a "Column E card" prior to the appearance of clinical signs, or after an animal is experiencing unrelieved pain and/or distress. Any new Column E animals or changes to existing Column E animals must be reported to the investigator.

Roles:

Investigator

- Place a Special Observations Card (Attachment 6503A) following induction of a model that is characterized as Column E or, in the case of spontaneous models, at the onset of clinical signs.
- Notify a CMS Veterinarian on the expected timeline for onset of clinical signs of pain and/or distress.

Animal Care Technician:

- Follow CMS SOP3402 for reporting new clinical signs in any animals, including those designated as Column E.

Veterinary Technician:

- Creates a Clinical Case Record or Column E Recording Sheet
- Notifies a CMS Veterinarian immediately if an animal is experiencing signs consistent with a pain score of 3, or if a Column E categorization has already been assigned.

Animal Care Technician III or designated CMS staff:

- Counts the number of Column E designated cages in the room twice daily (AM and PM) and completes the Column E Daily Recording Sheet
- Performs BID observations on all cages flagged with a Column E card and records findings on the Column E Observations Sheet.
- Frequency of observations may be increased at the discretion of a veterinarian.

- Documents any new abnormal observations on the Column E Observations Sheet and submit a new health report for the animal in the Progeny Database.
- Notifies Veterinary Technician or a CMS Veterinarian immediately if new clinical signs are noted or the condition has worsened.

Veterinarian:

- Places Column E card if indicated and approved per the ASP.
- Provides guidance to Technicians on Column E cases.
- Re-examines all Column E status animals weekly and determines if a change in frequency of observations is necessary; notifies Investigator with any changes. o Weekly observations are the default frequency; however, this may be increased at the discretion of the veterinarian.
- Records the date, clinical signs, pain score (see CMS SOP3402) and any treatment on the Column E Recording Sheet.
- Uses criteria in the IETC form or ASP, as needed, to determine whether the animal has reached the study endpoint.

When Study Endpoints are Reached:

- Once an animal meets a Column E study endpoint, as determined by a CMS Veterinarian, CMS staff will notify the Investigator by e-mail and phone that the Column E study endpoint has been reached. Inform the investigator that they must collect the required tissues or data within 30 minutes of notification or by the facility close of business (3:30 pm), whichever comes first, so that the animal is not allowed to suffer needlessly. If the investigator is unable to address the animal within 30 minutes, ask them to provide sample or data collection instructions. After 30 minutes or at the close of facility business, CMS personnel are authorized to euthanize the animal and place the carcass in the animal facility refrigerator for the Investigator to harvest tissues, collect data, or remove implants.
- If there is no response from the Investigator after 30 minutes (via e-mail and phone notification), the animal is euthanized and placed in the appropriate refrigerator. The investigator will be notified by e-mail about this action.

Removal of Column E Cards:

The veterinarian or designee will remove Column E cards from cages of animals that recover from research-induced illness, succumb, or are euthanized from Column E.

Weekends and Holidays:

Qualified CMS personnel perform twice daily observations and complete necessary paperwork as detailed above. If necessary, assigned

personnel or laboratory staff will perform additional observation(s) of the animals per previous agreement.

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.

The NIA utilizes the "NIH/ARAC Guidelines on Pain and Distress in Laboratory Animals: responsibilities, recognition and alleviation" as a fundamental guidance document in the regulatory requirements surrounding, methods to recognize, and intervention strategies, for painful or distressful situations.

Three groups may be required to be involved in monitoring animals for potential pain and distress.

1) **Animal care staff and veterinary technicians:**
   - Receive detailed training, to include pictures and quizzes, on how to identify animal pain and distress, what to do, and the ethical and employment responsibilities to do so.
   - Are also trained in expected conditions in ***aging animals*** which may necessitate treatment and veterinary consultation, include:

     1. Abnormal posture/kyphosis
     2. Unkempt hair coat
     3. Increased discharge from eyes and/or nose
     4. Skin lesions (e.g., dermatitis, necrosis)
     5. Altered respiration
     6. Slowed or atypical ambulation
     7. Decreased food or water intake
     8. Cutaneous tumors
     9. Chronic weight loss

2) **Veterinarians** – all CMS veterinarians are ACLAM-board certified, with extensive experience in assessing pain and distress in rodents. The clinical veterinarian has over a year of on-the-job experience with aged rodents and the backup clinical veterinarian has over 3 months' experience with this group of animals.

3) **Laboratory staff** sign a statement on their training and experience form attesting that they understand they must read and comply with all ASPs on which they work; all ASPs include

descriptions of the clinical signs signifying removal of animal from the study.

The NIA CMS staff have a specific set of procedures in place for monitoring USDA Column E animals. Please refer to section B.1.c.i.2) above for details.

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.

Subsequent to the daily standard animal health assessment, the research and veterinary care staff identifies deviations from anticipated experimental and spontaneous outcomes (e.g. phenotypic) described in the ASP. When any unexpected morbidity, moribundity, or mortality is identified by animal care, veterinary or laboratory staff, the Regulatory Veterinarian informally investigates the situation, to include communicating with the involved animal user, Principal Investigator, or others who have information, to determine root causes. She then reports back to the ACUC Chair and APD with preliminary findings.

For events that are related to study design issues, e.g., certain phenotypes or treatment groups are displaying unexpected clinical signs, the PI consults with the APD, AV and/or ACUC Chair to determine whether an amendment to the ASP and ACUC approval is required before continuing the study. The PI may amend the ASP to change the experimental endpoints to eliminate the previously unanticipated pain or distress or maintain original endpoints and propose a standard veterinary treatment plan and/or special husbandry practices to protect animal well-being. If efficient intervention would alter experimental results, then a scientific justification is required, and animals may be placed in USDA category E.

For events that appear to be due to protocol or other regulatory non-compliance, or in other situations as directed by the ACUC or APD, APD/AV will report preliminary information to the Scientific Director as an early notice. A meeting will then occur that includes the APD, ACUC Chair, Regulatory Veterinarian, PI and the primary individua(ls) involved, to continue fact-finding and to let the PI know that additional actions will occur. The Regulatory Veterinarian reports to the ACUC at the next convened meeting, although emergency meetings may be called if the situation requires it. The Regulatory Veterinarian may make recommendations to the ACUC for additional training or oversight; the ACUC will determine final actions, to include removal of the individual from animal, suspension of the protocol (following formal protocol suspension procedures), or other actions

as deemed necessary. The Scientific Director will be informed of these actions and may impose additional restrictions or consequences as he sees fit.

Events that appear to involve significant or ongoing noncompliance with animal care and use laws, regulations or policies will be reported to OLAW as required by PHS Policy.

Occurrences and any changes needed to address these occurrences must be reported during annual ACUC review; this includes details of animal treatment plans or changes in pain category.

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."

Section F of the NIH ASP form requires a description of experimental design and animal procedures, to include method of restraint. The ACUC policy on Restraint defines prolonged restraint, which must be scientifically justified and described in enough detail to show planning to minimize impact on animal welfare, and other (non-prolonged) restraint, which must be mentioned within the ASP.

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.

METHODS USED IN THE LAST THREE YEARS:

<u>189-LGG-2019:</u>

Sweat Test: A "sweat test" will be performed to determine the secretory function of sweat glands in the footpads of mutant and

WT mice. The mouse will be restrained by placement in a plastic cone, a plastic bag specially designed to easily restrain a mouse. After restraint, the volar surface of the paw is painted with a solution of 2g of iodine in 100ml absolute alcohol using a cotton swab and allowed to dry for 1 minute. The paw is then covered with a suspension of starch in oil (100g of starch powder in 100ml castor oil). In the presence of sweating, fine black dots will appear at the sites of sweat gland openings on the paw skin surface. The oil layer will prevent rapid evaporation of the sweat and will retain the black color long enough for photographs to be taken, which will be done immediately as the staining develops. The pattern of staining begins to develop 1 to 2 minutes after treatment, usually reaching maximal development by 10 minutes. Therefore, the maximum amount of time a mouse would be restrained in these cones is approximately 15 minutes. The mice will be closely monitored throughout the procedure for signs of extreme stress or overheating. The effective way to monitor the mice during the procedure is to closely observe their breathing behavior. If the mice breath slowly and deeply, they should be released immediately from the cone. Sweat test will be performed once per animal. From our experiences during past 18 years, a special temperature control is not necessary, and we do all procedures in the room temperature.

191-LCS-2019:

Blood pressure (BP) and heart rate will be monitored every week via tail cuff (IITC Inc/Life Science Instrument). For that, rats will be placed into a plastic cylinder holder and BP will be recorded for 10-15 min (2-3 sets of measurement by 2 minutes each set with the rest between measurements by 4-5 minutes). Body weight will be recorded twice a week. Animals will be trained to stay in a restrainer for 10-15 min during the blood pressure measurement (usually it takes 2-3 training sessions: during first session animals will be introduced to the restrainer for a few minutes, during second session animal will stay inside for 5 minutes without tail cuff inflation, during third session animal will stay inside restrainer for 10-15 min with inflation of tail cuff (1 inflation per min) with blood pressure recording). Tail cuff will be inflated, and the sensor will record the signal. (See Appendix 360).

341-LCS-2019:

Systemic blood pressure (SBP) and heart rate (tail cuff technique) will be measured before treatment and monthly thereafter. The IITC MRBP sensor technology to measure the rat

tail blood pressure. Note, all rats will be first acclimated to the SBP measurement device for 3 days (these data will be discarded). Importantly, each day, ten acclimation cycles will be followed by fifteen measurement cycles, which will be averaged to obtain the average SBP of that rat on that day. Volume pressure recordings (VPR) VPR are clinically validated and provide close to 100 percent correlation with telemetry and direct blood pressure measurements. This VPR technology is based on rat tail blood volume and can actually measure the diastolic blood pressure. The software allows us to continuously view data in real-time, and archive and export rat blood pressure information.

405-TGB-2021:

Blood pressure monitoring as per Appendix 360 and Metabolism in Mice Using the CLAM System under Exercise (Appendix 361).

407-LBN-2020:

Physical restraint -- For stimulation session durations under 30 minutes, rats are either physically or chemically restrained. Physical restraint may include wrapping the rat in a towel and securing by hand or placing the rat in a small chamber to restrict movements. The chamber is adjustable with a darkened cone for the rat to place its head. A bright light will be placed over the rat to encourage placement of their head in the cone. Rats may be subjected to either water restriction (following procedures specified in ACUC Appendix 339) or food restriction (following procedures specified in ACUC Appendix 314) to provide motivation for keeping their head in the tubes. Depending on the restriction (food or water, never both) either water or sweetened cereal will be delivered at the tip of the cone to encourage rats to maintain their head inside. Four to ten days of training will be provided to habituate the rat to the chamber and cone, and to reduce the stress response.

Other physical restraint procedures are also used in this protocol, varying in degree of head restraint:

The maximum duration of restraint will be 30 minutes per session, and rat will be habituated to the restraint by increasing the restraint duration over a period of 1-2 weeks. For example, after a 5-minute restraint session on the first day, restraint duration would be increased in 5-minute increments daily until the maximum of 30 minutes is reached. The actual restraint time and rate of increase will be individually tailored to each rat depending on the rats' stress level on the previous day. Indicators of stress include vocalizations, defecation, urination, and struggling to escape.

Rats will be lightly anesthetized with isoflurane via an induction chamber with a vaporizer, (as described in main ASP), 1-5% to effect, so that the rat can be quickly secured into a soft, cloth like wrap and then placed into either a tube or a form fitted restraint with adjustable straps to further restrict movement. The rat may also be restrained by either just the wrap, or just the form fitted restraint or tube.

To further restrict head movement additional straps, or a cone to fit snuggly over the rats' snout may be added to the restraint device. Other modifications may include the addition of a bite bar (similar to that in a surgical stereotaxic or in rodent restraint devices used in imaging studies) flexible plastic blunted ear bars, and devices to restrict moment of the upper torso. Lidocaine paste (2%) may be added to points of mechanical restraint, e.g., bridge of the nose and ear canals to minimize any pain or discomfort.

In all instances, rats will be briefly anesthetized during the time it takes to place them in the restraint. Sufficiently securing the head and upper torso to reduce movement is designed to eliminate self-damage that could possibly occur upon recovering from anesthesia. The goal is to have the rat fully recovered from anesthesia during the TMS stimulation session. The exact hardware used to restrain the rats may differ slightly from the above description, but the experience of the rat will not differ.

Chemical restraint -- For stimulation session durations over 30 minutes, a chemical restraint will be used.

415-TGB-2021:

Hind limb suspension: Atrophy of the hind limb muscles will be induced by unloading of the mouse hind limbs through taping of the animal tail to a wire suspended from the top of the cage at an angle of 30 degrees. This will result in a 50% load on the front forelimbs. This apparatus allows unrestricted hind limb movement and freedom to move around the cage in any direction using its forelimbs for locomotion. Published literature using this model including an extensive review by Morley-Holton and Globus (J. Appl. Physiol 92: 1367-1377) from Life Sciences Division at NASA Ames Research Center suggests that it is an effective means of inducing muscle atrophy that is safe and tolerated by the animals for up to 3 weeks. The review by Morley-Holton and Globus indicates that animals tolerate the procedure well, are not overly distressed once acclimated and do chew at themselves or the unloading apparatus. A brief description of taping the mouse for the hind limb suspension protocols is: The tail is cleaned with 70% ethanol and allowed to dry. A thin strip of traction tape is fished through the wire and attached to the base of the tail just

above the hairline of the mouse. The tape is stuck to the tail from the base to ~1/3 to 1/2 the length to the end of the tail. The traction tape is secured via 2 thin strips of surgical tape, one at the base and one approximately 1/3 to 1/2 of the length to the end of the tail. The tape should be loose enough to allow for circulation. The area near the base of the tail can be wrapped with gauze to prevent the mouse from peeling off the traction tape.

445-LCS-2019:

BLOOD PRESSURE MONITORING (APPENDIX 360): Blood pressure measurements will be performed at three age time points (2, 8 and 12 months) via tail cuff (IITC Inc/Life Science Instrument). For this procedure, mice will be placed into a plastic cylinder holder with tail cuff for 15 min while machine records blood pressure and heart rate. Training or habituation to procedure will occur at a maximum of 1 week prior to readings for approximately 3 sessions before measurement. Actual measurements will consist of a minimum of 3 separate readings at least 24 hours apart. If any distress is observed animals will be removed immediately. The technician has previous experience using the IITC tail cuff device on mice and rats.

448-LCS-2020:

Blood pressure and heart rate will be monitored every week (Experiment A) or every month (Experiment B) in conscious animals via tail cuff using the MRBP System (IITC; Woodland Hills, CA). The procedure will take 10-15 min per animal (Appendix 360). Mice will be restrained in the transparent plastic tube with the holes. The restrainer allows animals to move, but not to turn. The animals learn very quickly (after 1-2 training sessions) to stay in the restrainer for up to 15 min without moving with their tail in the sensor devise.

Failure to Acclimate: Animals that fail to acclimate properly are removed from that arm of the study or may be completely removed from study. Scientists at the NIA understand that data obtained from stressed animals, especially data such as heartrate, blood pressure and other physiologic measurements, will be skewed in stressed animals.

Clinical Issues: Resulting clinical issues in any of these situations are referred to the veterinarian per normal procedures.

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.

Regardless of classification (i.e., minor or major per the Guide) all surgical procedures, and especially any that are repeat procedures (even if minor in classification) are reviewed by the ACUC to determine their scientific value as well as potential impact on the animals.

Multiple major surgical procedures are not permitted unless scientifically justified and approved by the ACUC. Multiple major survival surgeries are not allowed on animals on separate protocols.

Multiple major survival surgical procedures can only occur within the same study and must be described and justified in section G of the NIH ASP form. The ACUC evaluates this information and determines whether or not the proposed surgeries are consistent with the recommendations of the Guide, PHS Policy, and the AWRs.

The ACUC apply these criteria for approval of proposed multiple survival surgery:

- The surgical procedures must be related components of the research proposal.
- Multiple major survival surgeries may be done if needed for clinical reasons.
- Animal well-being must be considered, and outcomes evaluated.
- Cost savings is not an adequate reason for performing multiple procedures.

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.

There are several studies in which rodents undergo multiple surgery procedures one week to two months apart. These are all considered to be related components of the research project.

191-LCS-2019 (Rats):
Minipumps (ALZET), filled with vehicle (30% alcohol, 20% DMSO; pharmaceutical grade for both chemicals), or MBG or ouabain solutions (10-100 ug/kg/day for both steroids; pharmaceutical grade for both compounds), will be implanted subcutaneously (SOP 315), usi g the surgical procedure,

described in SOP 316. The surgery for the minipump implantation will take 10-15 min per rat. The life-time of minipumps, which we are planning to use is 4 weeks, so if a prolonged treatment is required, the minipumps will be replaced every 4 weeks two more times (total duration of the treatment is 12 weeks). Possible complications: rats may scratch the scar, so the topical application of antibiotic and anti-histamine treatment is possible. The minipump is a cylinder of an approximately 1x2 cm. The presence of minipump under the skin it is not painful or disturbing.

309-LCS-2021 (Rats and mice):

Minipumps (ALZET), filled with Angiotensin II, MBG, ouabain, losartan or lisinopril, will be implanted subcutaneously (Appendix 315), using the surgical procedure described in Appendix 316. The surgery will be performed under Isoflurane anesthesia. The surgery for the minipump implantation will take 10-15 min per animal. Possible complications: animals may scratch the scar, so the topical application of antibiotic and anti-histamine treatment is possible. The minipump is a cylinder of 5x1.4 cm for the rats (Alzet, model 2ML4) and 0.3x1 cm (Alzet, model 2006) for the mice. When it is placed under the skin, it looks like a bump, which can move with a skin. The presence of a minipump under the skin is not painful or disturbing. In the case of unexpected problems, please, contact the PI or an alternative co-investigator. Some animals will have two survival surgeries in order to increase the treatment time (up to 8 weeks). The life time of the minipumps is 4 weeks, thus after 4 weeks of experiment the used Alzet minipump will be removed, and a new pump will be implanted subcutaneously, as described above.

438-TGB-2019 (Mice):

The only surgeries that will be undertaken involve the implantation of and the subsequent removal of, subcutaneous mini-osmotic pumps that are used to deliver the drug Exendin-4. The procedure is minor and takes less than 5 minutes from start to finish. Due to the short time required for the pump implantation/removal, in the past we have successfully used the gaseous anesthetic Isoflurane administered via use of a vaporizer (per CMS SOP 6401 and 6402). All surgical equipment will be autoclaved prior to use, between animals the instruments will be sterilized in a glass bead sterilizer. The pumps we will use are active for 7 days they will be removed immediately after the 7 days have elapsed. The removal of the pump will involve a second minor surgery. After each surgery, the incision will be closed with sterile staples which will be removed 7 to 10 days after the surgery.

443-LCI-2019 (Mice):

Micro osmotic pumps (ALZET, Palo Alto, CA) containing vehicle or S961 (2.5 – 20 nMol/week/mouse) or Leptin (5ug/ul) will be inserted subcutaneous following CMS SOP 6522, ACUC Appendix 315,and the guidelines of ALZET (http://www.alzet.com/resources/technical_tips.html). Skin closure methods will follow CMS SOP 6511. It has been well documented by Blum et al. that insulin resistance and diabetes is reversible within 1 week of the removal of the micro osmotic pump containing S961. A second survival surgery may be performed to remove the ALZET pump after administration of S961 to reverse diabetes and examine the re-differentiation/re-maturation of beta cells in tissue specific knockout mice. The surgery and post-operative care would be performed exactly as described above with the exception that we would be removing the subcutaneous pump. The mice would be euthanized 7-10 days following the removal of the pump. We expect no complications from this second surgery as removing the insulin receptor antagonist should alleviate any diabetic symptoms. Blum et al. Reversal of β cell de-differentiation by a small molecule inhibitor of the TGFβ pathway. Elife 3:e02809, 2014.

464-LNG-2021 (Mice):

Animals are prepared via stereotaxic surgery (this portion is not repeated). The animals then undergo one of the following sets of surgeries (excerpted information follows):

2. Intracranial AAV injection: The AAVs (0.1-1 µl, titer 2-10 x 10e12 gc/ml) will be injected to the brain areas of chosen coordinates using a 2 or 10 µl Hamilton syringe. Liquid tissue adhesive is applied to the wound for a quick and painless closure.

3. Intracranial rabies virus injection: This procedure is composed of 2 steps of injections. The first step is the injection of AAVs, which is described as aforementioned. Since the mice will receive another surgery, the wound will be sutured instead of using liquid adhesive. The second step is the injection of pseudotyped G deleted Rabies virus (EnvA). Two weeks after the injection of AAVs, 0.4 - 1 µl of pseudotyped rabies virus (1.0x10e7 pfu/ml) will be injected to the same or nearby location of the brain. Then the wound can be closed with liquid adhesive and treated with the triple antibiotic ointment.

4. Cannula and fiber optic ferrule implantation for optogenetic stimulation: Immediately after injection of AAVs encoding channel rhodopsin, mice will be permanently implanted with cannula and fiber optic ferrule. Two holes will be drilled into the

skull to insert the anchoring screws that will be used to secure the head cap. Epoxy and dental cement will be applied around the implanted cannula or optical fiber. After three weeks recovery, optic fibers will be attached on mouse skull above the fiber optic ferrule. In this way, neuronal activity can be modulated by blue laser light pulse through the optic fibers in behaving mice.

5. GRIN lens implantation for brain imaging: At least 10 days after injection of AAVs, mice are permanently implanted with GRIN lens. A hole will be drilled into the skull to insert the GRIN lens. Epoxy and dental cement will be applied around the implanted lens. After three weeks recovery, a mini-Scope will be mounted onto the dental cement covering the mouse skull above the GRIN lens through an external attachment. No additional surgery is required. A detailed protocol is attached in the Supplement Section.

6. Optic probe implantation and fiber photometry imaging: The mice will experience two surgeries. The first is to inject AAVs carrying genetically encoded fluorescence sensors in the designated brain regions. After the initial injection of AAVs for 4 to 6 weeks, the mice will undergo the second surgery for the optic probe implantation. The targeted brain regions for the two surgeries can be the same or different depending on the individual experiments.

481-LGG-2022 (Mice):

Repeat muscle injury – while this technically does not qualify as surgery, it is included because it is a repeated injury that causes prolonged effects in the animal. First, the host mouse will be anesthetized with isofluorane and the tibialis anterior (TA) muscle of the hindlimb located. With a syringe parallel to the length of the TA muscle, a syringe will be inserted just below the kneecap and pushed in until the tip has reached the bottom of the TA near the ankle. The syringe will be slowly pulled out while injecting so that the injection volume is released along the entire length of the TA muscle. This ensures the most consistent TA muscle injuries. A maximum of 5 injection sites along the TA muscle will be used. The muscle will be allowed to recover for 3 weeks before a second injection. In this way, subsequent injections will be performed to maximize the replicative divisions of transplanted MuSCs (Hall et al., 2017).

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.

The NIA adheres to the NIH ARAC, "Guideline for Diet Control in Laboratory Animals". Food and fluid restriction are used for various protocols within the NIA; procedures are described within ACUC Appendices 301.1 and 314 as well as in the individual ASPs. ASP-approved procedures take precedence over Appendix procedures.

Food Restriction (General): The following dietary restriction protocols may be used for heath maintenance or for experimental goals:

1. Every-Other-Day Feeding (aka Alternate-Day Fasting): The dietary restriction regimen involves an alternate day feeding schedule in which food is provided ad libitum on one day and the following day the animals are fasted.

2. Isocaloric Pair-Feeding: In this feeding regimen, animals are provided a daily allotment of food (equal in calories but may differ in grams of food) to that of the ad libitum fed control group. Animals receive the daily allotment of food once per day at a predetermined time, in the hopper. Each day, any food remaining is removed from the hopper and replaced. Food remaining is recorded daily on a monitoring sheet.

3. Limited Daily Feeding: Animals are provided access to food during only a limited time window of 1 hour per day. This feeding regimen is a modification of our every-other-day feeding protocol that extends lifespan in rats and mice.

4. Calorie restriction (CR) Feeding: Animals are fed 20-40% less calories than animals in the ad libitum (AL) fed control group. In this feeding protocol, all animals have body weight measurements performed at least weekly (unless specified otherwise in the ASP). AL animals have their food consumption measured and from this the reduced caloric intake (20-40%) is determined every other week and

adjustments of food are made until the animals' body weights plateau (around 16-18 months of age, or after 10-15 weeks on diet), at which point the daily food allotment for CR animals is fixed for the remainder of their lives. CR animals are fed daily between 8-10am or 12-3pm, depending on the study, with the food allotment provided to the animal on the floor of the cage, and not in the hopper.

A step-approach is used for restriction of animals so that they can adjust to reduced caloric availability. If performing 40 % CR, start with 20 % CR for week one, 30 % CR during week two, and proceed to 40 % CR at week three. If animal health declines, extend step-down time or consider a lower percentage of caloric restriction. For each restriction regimen the body weights of the animals will be recorded at least weekly (unless specified otherwise in the ASP), and animals observed daily for signs of morbidity, such as, emaciation or not eating their daily ration. Any restricted animals with food left over from the previous day have the remaining food allotment removed. The PI will be notified when food is remaining for restricted animals.

Fluid Restriction: Fluid restriction is typically only used for behavioral testing motivation.

Justification – Experimental Studies:

Lifespan: Dietary and calorie restriction, as described in NIA protocols, are the primary means of increasing lifespan of mice and rats and are known to greatly reduce the incidence of age-related cancers and improve the overall health in these rodent species. Animals on dietary and calorie restriction are carefully monitored with regards to food and bodyweight. As part of longevity studies, animals undergo bodyweight measurements at least weekly and food allotments for restricted animals are adjusted to ensure adequate nutrition. These dietary regimens are well known and well documented. The beneficial effects of calorie restriction were first reported in 1935. We have studied the effects of calorie restriction on aging for the last 30 years in this laboratory and have observed no significant signs indicating that the animals are in pain or distress. Occasionally, we do have animals that experience health issues. In these cases, animals will be referred to a CMS veterinarian for consultation on treatment and/or euthanasia. Endpoints will follow those approved in the specific ASP.

Experiment-specific impact: Additional ASP justifications for dietary and/or calorie restriction include assessing the impact of specific

nutrients in the onset or severity of age-related pathology, or the impact of these restrictions on animals that already demonstrate age-related pathology.

Behavioral impact: The impact of dietary restriction on behavior may be measured for some studies. This is a separate justification from the need to motivate animals to perform on behavioral tests described below.

References: Ingram DK, Weindruch R, Spangler EL, Freeman J , Walford RL. (1987) Dietary restriction benefits learning and motor performance of aged mice. 42:78-81.

Duan W, Lee J, Guo Z, Mattson MP. (2001) Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. J Mol Neurosci. 16:1-12.

Curtis J, de Cabo R. (2013). Utilizing calorie restriction to evaluate the role of sirtuins in health span and lifespan of mice. Methods Mol Biol. 1077:303-311.

NIH ARAC Guideline for Diet Control in Laboratory Animals https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b7-diet_control_12-1-2017.pdf

Justification – Behavioral Testing: NIA studies may use a variety of behavioral assays, many which rely on the motivation of the animal to make a behavioral response (e.g., dig in a cup of sand or walk down an arm of a maze) to obtain a food (e.g., sweetened breakfast cereal) or a liquid (e.g., water, milk) reward. Restriction of food or water is typically maintained for the duration of the task. Extensive testing has shown that mice maintained at 80-90% of their free feed weight are optimally motivated to perform these types of behavioral tasks and that the mice suffer no ill effects from the reduction in caloric intake. Investigators wishing to use food or water restriction as a means of motivation for behavioral testing studies are referred to the ARAC, “Guidelines for Diet Control in Behavioral Animal Studies”.

Species involved:

- Mice
- Rats
- Nonhuman primates (we do not currently house or hold NHPs)
- We do not food or fluid restrict rabbits

Length and type of food/fluid regulation: Rodent food restrictions may last from 1 week up to 6 months or longer, depending on the goals of the study involved. For specifically behavioral testing, this length of restriction may not be needed; however, it may come after the animal has already been on a restriction study for a number of months and therefore the animal will have been on this restriction for months.

Animal health monitoring procedures and frequency (e.g., body weight, blood urea nitrogen, urine/fecal output, food/fluid consumption) and methods of ensuring adequate nutrition and hydration during the regulated period:

At a minimum, laboratories must maintain records, which document weekly body weights (or more often if requiring greater food restriction) and daily food/fluid consumption, hydration status, and any behavioral or clinical changes. These records should be available for examination by the veterinary staff and ACUC if necessary. Furthermore, appropriate contact information should be provided in cases where it is not obvious that animals have been provided their daily ration. If CMS is unable to verify feeding/fluid provision and is unable to reach the appropriate laboratory contact, food/water will be given to animals. All details regarding food or water restriction must be included in the approved ASP. In addition, the following are recommended unless otherwise modified by experimental needs or the ASP:

- Rodent food restriction:

    1. Gradually reduce animals to a target weight and acclimate to the feeding schedule to mitigate the stress response; body weight should not be reduced more than 10% per week.
    2. Maximum weight loss under diet restriction should not exceed 20% of initial body weight.
    3. Daily ration should contain at least 30% of minimum caloric requirements when establishing a target weight and should be provided at the completion of testing.
    4. Water should be provided ad libitum.
    5. If any animal appears lethargic or abnormal as a result of the food deprivation, that animal should be immediately removed from the experiment and placed on an ad-libitum feeding schedule. 6) At the end of the behavioral testing, animals should be placed on an ad-libitum feeding schedule.

- Rodent water restriction:

1. Prior to fluid restriction, body weight and fluid intake should be recorded daily. Once a baseline fluid intake has been established on a given task, each animal should be allowed to earn fluids to satiety or to a level approved in the ASP.
2. Gradually limit fluid intake over a several day period.
3. Additional access to fluid should be provided on days when research procedures are not scheduled.

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.

The NIA ACUC follows the NIH ARAC, "Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals" in its review of ASP and amendments. These guidelines incorporate the Guide and Animal Care Policies requirements for pharmaceutical-grade drug use, as well as justifications for exemptions from these requirements.

The use of non-pharmaceutical grade compounds should be based on (1) scientific necessity, (2) non-availability of an acceptable veterinary or human pharmaceutical-grade compound, and (3) specific review and approval by the ACUC.

When reviewing a proposal to use non-pharmaceutical grade compounds the ACUC considers animal welfare and scientific issues related to the use of the compounds, including potential for contamination, safety, efficacy, and the inadvertent introduction of confounding research variables.

For all compound use, the ACUC considers the grade/purity being proposed, the formulation of the final product, and issues such as sterility, pyrogenicity, stability, pH, osmolality, site/route of administration, pharmacokinetics, physiological compatibility, and quality control.

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.

N/A

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

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

Some rodents and nonhuman primates are reused if not euthanized at the end of an experiment. Care is taken to obtain the full history of animals prior to transfer. Some excess breeding rodents and unused replacement sentinels are transferred to the training ASP and used for training purposes.

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.

The animal training protocol may use animals transferred from other protocols. This allows for reduction of overall animal use on the training protocol, which provides critical skills development for animal users.

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.

While animal adoptions have not occurred in the past, we recognize the need to have a policy in place for any potential future situations that may allow adoptions to occur. For that reason, an adoption policy is currently in progress it is based on an existing policy in effect at the Division of Veterinary Resources (DVR), NIH. It has not been officially approved as of the submission of this PD but may be approved prior to AAALAC site visit.

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 primary goal of the Post Approval Monitoring (PAM) program is to monitor congruence between laboratory practice and the approved Animal Study Proposal (ASP), so as to ensure compliance. Factors to be monitored include, but are not limited to, the number of animals used, proper training of staff, experimental procedures being performed, maintenance of accurate records, and use of appropriate analgesia and anesthesia. In addition, the PAM program may be utilized as one method to monitor the overall animal program and to provide input to the Animal Care and Use Committee (ACUC) regarding processes, identifiable risks, and to foster a positive research culture.

An effective PAM program should also strive to keep investigators aware of new policies, regulations, and guidelines that may affect protocol compliance and laboratory "best practice" and we do this through monthly AV meetings with each lab, as well as through our ACUC newsletter.

Finally, the program should strive to establish a collaborative partnership with investigators to promote animal welfare, a culture of compliance, and to provide open forums of communication between investigators, the ACUC, and the staff of the Comparative Medicine Section (CMS).

According to the eighth edition of the Guide, PAM activity can be carried out by various individuals including, ACUC Office staff, ACUC members, veterinary staff, animal care technicians, and others. Therefore, the participation and responsibilities of the following groups are defined as follows:

ACUC Members: All ACUC voting members participate in PAM activities *via the ASP re-review and annual review process, semiannual facility inspections, and semiannual program reviews.*

Animal Care & Veterinary Staff: This includes a number of people at various levels of responsibility including the Attending Veterinarian (AV), veterinary staff, management staff, and animal husbandry staff. These individuals carry out a large number of duties related to animal care and use and are thus active participants in the PAM program.

Specific activities include, but are not limited to: *routine animal care and observation of animals on active studies that can allow for verification that animal procedures and clinical conditions conform to what is approved in the ASP, to include endpoint monitoring; and in-lab assessment and re-training of technical skills as directed by the ACUC, requested by PIs, or otherwise arranged.*

Regulatory/Compliance Staff: This includes the Regulatory Veterinarian, the ACUC Program Coordinator, and the Compliance Specialist. The Regulatory/Compliance staff is responsible for *periodic review of approved protocols for congruence, for review of requests for technical services, for monitoring the number of animals used on each ASP, and for review of animal import and export requests.* Regulatory/Compliance staff also ensure *completion of ACUC action items related to approval of ASPs and amendments, issues of noncompliance, approved pilot studies, and other actions taken by the ACUC.*

A number of PAM activities are detailed below. Some of these are considered "informal PAM processes" and performed on a continual, rolling basis. Others are more formal and are performed periodically, or on an as needed basis.

Ongoing ASP review: All approved ASPs are subject to periodic administrative review. This occurs annually, at the ASP approval anniversary. This process helps to ensure protocol compliance, and acts as a check to make sure no changes have occurred without approval. Animal welfare concerns and unanticipated events can also be discussed at this time. This review is performed at a convened meeting of the ACUC. In addition to annual review, all ASPs are subject to three-year renewal, at which time a new ASP is required, and thus all animal activity is reevaluated based on current policies, regulations, guidelines, and current best practices.

Semiannual facility inspections: These inspections are performed by ACUC members with the assistance of the facility management staff. Although aimed mainly at facilities and animal care and use program policies and procedures, these inspections also aid ASP compliance review in the following manner: a) allow for review of training records, b) allow for review of compliance with occupational health requirements, c) monitor compliance with facility-related issues regarding equipment and processes, d) monitoring of protocol-related drug storage and usage, e) monitor compliance with guillotine procedures (review of maintenance/procedure logs), and f) monitoring of animal health and welfare on specific protocols.

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

Not less frequently than every six months, in April and October, the ACUC reviews the animal care and use program. We utilize a NIH-specific semiannual self-assessment checklist

Program review occurs at the ACUC meeting immediately following the facility inspection (Appendix 10). The Committee walks through the checklist referencing materials such as policies, SOPs, and other items.

In addition, an ACUC subcommittee may also review SOPs, policies, protocols, appendices, training records or other documents associated with the animal care and use program prior to the full committee review, to augment the overall Committee program review.

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, in April and October, the ACUC inspects the animal facility using a team of at least two voting ACUC members.

In addition, any areas outside of the facility where survival procedures are conducted (e.g., surgery, MRI, behavioral assessments, etc.) are inspected every six months by a team of at least 2 voting ACUC members.

Areas outside of the facility where only euthanasia is performed are inspected at least annually by a team of at least 2 voting ACUC members.

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.

This is not applicable since, as a Federal research facility, the NIA is not subject to routine USDA inspection.

(An Annual Report to USDA is required because of our use of rabbits and, occasionally, nonhuman primates; NIA data is rolled into a central report that is compiled and submitted by the NIH OACU).

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

Post-approval monitoring activities also include the following, not mentioned previously:

Animal use space walk-throughs: The Regulatory Veterinarian reviews all animal procedure space monthly, and Animal Facility Management reviews all animal holding space monthly, with additional external (non-NIA) animal space review every quarter. The ARPM reviews various areas on a rotating basis throughout the animal facility monthly, to provide quality control over animal care and use processes as well as contractor performance in providing certain animal care services. This provides additional oversight for appropriateness of animal holding and use but may also provide insights into protocol-specific situations, to include animal conditions on certain protocols.

Ongoing oversight by animal facility staff: Animal-related activities that occur within the CMS facilities are subject to routine monitoring and evaluation by the CMS staff. This includes, but is not limited to, a) daily observations of animal health and welfare, b) periodic observations of standard procedures, and c) monitoring of compliance with signage, PPE, and standard laboratory practices.

Other (random) walk throughs/discussions: One mechanism for developing a "culture of compliance" is to be available to investigators and to become familiar with their research. In that regard, the APD, ACUC and veterinary staff may periodically visit laboratories and procedure rooms to check in with investigators and their staff, to answer questions, or to disseminate information.

Formal "for cause" protocol monitoring: In some cases, described below, increased PAM monitoring is necessary. These activities are generally performed by ACUC members.

- *Specific instances of noncompliance*: Depending upon the nature of the offense, PAM activity could be limited to a particular individual or procedure, or could be broader, encompassing a whole ASP or laboratory. In general, a subcommittee of the ACUC will investigate and propose sanctions and/or retraining requirements, which would be subsequently approved by the full ACUC.

- *Specific requests (other than noncompliance):* At times, the ACUC, veterinary staff, or others may raise concerns related to protocol issues that are not yet to the level of noncompliance. It is critical that these issues be addressed in a timely manner so that more serious issues do not arise. These will be handled on a case-by-case basis in much the same manner as noncompliance issues.

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

An ACUC policy regarding reporting ACUC concerns lays out the mechanism of reporting and possible consequences of substantiated claims.

Anyone in the NIA/IRP who has concerns regarding the care and use of animals is strongly encouraged to report the concerns. Concerns can be reported by the following methods: telephone, email, and/or anonymous online submission form. Anonymity of the individual initiating the concern will be respected, if requested and if possible. The NIH does not tolerate any reprisal against an individual who has come forward with concerns involving the care and use of animals.

In addition, the NIH Deputy Director for Intramural Research (DDIR)/IO policy memo, "Communicating Animal Care and Use Concerns within the NIH Intramural Research Program" is prominently posted throughout the vivarium and laboratories – these contain points of contact within the NIH for reporting concerns and the process whereby this is accomplished.

All animal welfare concerns are reported to the ACUC chair and the APD and subsequently investigated by a subcommittee of the ACUC. Depending on the nature and severity of the welfare concerns, the incident may also be reported to the OACU by the APD, Regulatory Veterinarian, or ACUC Chair, as appropriate, through the ACUC Coordinator. The OACU, which reports to the IO, ultimately decides if the concern is reported to the OLAW. The subcommittee either presents its findings at the next scheduled ACUC meeting or calls for an emergency meeting specifically to address the welfare concerns. Based on the investigation results and ACUC deliberations, if the claim is substantiated, the ACUC will notify the OACU and take all necessary actions to prevent future similar events. The OACU will report to the OLAW and AAALAC, if necessary.

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 NIA's CMS Disaster Plan describes a number of key points with regards to disaster management:

- Chain of command
- Animal-related procedures to include triage/euthanasia and decision - makers for euthanasia
- Essential personnel designation

Chain of Command / institutional components and personnel which would participate in the response:

- Government Facility Management:
  - Dr. Kathy Perdue, Animal Program Director (APD) (office) Redacted by agreement

- Redacted by agreement
- Redacted by agreement (office)
- CMS animal care and veterinary staff ***(all are considered "essential personnel")***

- Emergency Services:
  - Baltimore City Fire and Police (EMERGENCY) 911
  - Baltimore City Fire and Police (Non-Emergency) 410-396-2499
  - NIA Redacted by agreement Security Redacted by agreement
  - NIA Safety Office Redacted by agreement
  - NIA Safety 24/7 Hotline Redacted by agreement
  - GSH Facilities Operations Redacted by agreement

Default leadership for any building operations involving animals is Dr. Perdue, the Animal Program Director/AV.

Training: Training occurs for this plan at both the OACU/NIH/NIA leadership levels as well as at the organizational/animal care staff level.

Emergency Classifications:

Level 1: Minor Emergency - Examples: short-term power outage, weather that briefly prevents personnel from reaching campus. Will be handled by animal care staff, investigators, facility management and may require GSH Facility Operations assistance. No outside help required.

Level 2: Emergency Requiring Outside Assistance - Examples: fire, localized flood, biohazard spill. Animal care staff, investigators, and facility management may be involved in animal care. Level 2 emergencies will also require outside assistance from other departments and/or city emergency response personnel. For instance, a bomb threat may involve NIA Security, Baltimore City Emergency Medical Services, and Baltimore City or County Law Enforcement. Level 2 emergencies typically involve a single facility or building. Damage assessment will be conducted by NIA Safety, Redacted by agreement Manager and emergency personnel and reported directly to Facility Operations. While level 2 emergencies may be extensive, NIH/Bethesda and community resources are generally readily available to assist the Baltimore campus.

Level 3: Catastrophic Event - Examples: major earthquake, hurricane. Everyone is aware and outside assistance may be overwhelmed. Priority for human safety may not allow for animal care. Level 3 emergencies are infrequent and catastrophic and will likely exceed the capacity of local emergency response teams. Laboratory animal staff may need to respond to the crisis for several hours or longer without outside assistance. As in level 2, the Redacted by agreement Manager or designee staff member on site with highest seniority will communicate damages directly to

Facility Operations, NIA Safety, APD and Redacted by agreeme Security. Emergencies of this level are under the direction of the APD and or NIA Scientific Director. The CMS staff will follow all directives and instructions issued by these authorities.

Descriptions of provisions for addressing animal needs and minimizing impact to animal welfare: Response is determined by the situation. When human safety is not put at risk, animal care continues, described as follows in our Disaster Plan (excerpts):

Triage methods/Euthanasia: Euthanasia will be a last resort and will be conducted under the direction of the APD and veterinary staff.

General Disaster Protocol for Animal Identification, Triage, Transportation, or Euthanasia:

1. Identification of Genetically Distinct or Irreplaceable Animals: ***Aged animals on longevity projects and genetically distinct strains unavailable elsewhere are considered unique resources and are therefore*** prioritized for removal in the event of a disaster. The APD and Facility Management maintain a generalized priority listing of these animals and their locations. This list is considered a working document and is revised as necessary with changing research needs. Investigators are encouraged to utilize a commercial cryopreservation program as another means of resurrecting strains in the event that facility wide euthanasia is employed during a catastrophic event.

2. Triage of Animal Populations: In the event of a major disaster affecting a localized group of animals or a building-wide disaster, injured or affected animals will be triaged by trained animal care personnel and/or emergency veterinary staff as long as human safety is not compromised. Those with life-threatening injuries or conditions not amenable to recovery will be euthanized on site by trained personnel under the direction of the APD or veterinary staff.

3. Relocation/Transportation: Traditional laboratory rodent, rabbit colonies and primates will be moved to other like animal-approved facilities if possible.

4. Anesthetized Animals: Research animals that are under anesthesia during a disaster may require immediate euthanasia if staff do not have time to recover the animals in a safe environment.

5. Mass Euthanasia: If it becomes necessary to euthanize specific colonies, rooms, or the entire facility, trained personnel will accomplish this ***under***

the direction of the APD or veterinary staff. Euthanasia via overdose of CO2 or inhalant anesthetic will be utilized for rodents when possible. Water bottle inversion may be necessary as means of humane euthanasia. Euthanasia of larger species (including rabbits) by barbiturate overdose will be conducted.

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 of the rooms within the vivarium have individual temperature and humidity controls. The Building Automation System (BAS) is a stand-alone, PC-based, distributed digital control system that provides automated control and monitoring of the room temperature and humidity. The BAS electronically records temperature and humidity at frequent intervals and archives several weeks of data for long-term recall. There are two BAS Operator Workstations; one in the building engineer's office to monitor and control the entire building and one in the ARPM's office Redacted by agreement to control and monitor the animal facility. The system provides electronic and hardcopy reports on demand that summarize environmental conditions and alarms generated by the BAS.

The BAS notifies building engineers, the APD, and animal care supervisors via telephone of excursions to alarm points after a 30-minute hold period.

The air temperature and humidity are also monitored at the room level and recorded by the animal care staff. They report any readings that are out of range to Facility Management.

Animal Facility Alarm Parameters: the NIA and NIDA animal programs and members of the Office of Research Facilities (ORF) and George S. Hall, Inc.

Group (GSH) management identified and implemented standardized alarm parameters in a two tier system that specifies notification requirements in the face of critical HVAC parameter alarms, such as high temperatures or low air flow, that could greatly impact animal health after hours when the animal program staff are not present.

The Building Automation System (BAS) at the Redacted by agreeme. is monitored at the ORF/GSH, Central Trouble Call Desk at all hours. During normal working hours, 7:00 am to 3:30 pm, Monday through Friday, the animal facility's staff is responsible for monitoring their facility; after-hours, 3:30 pm to 7:00 am, and all day on weekends/holidays, monitoring is performed by the Central Trouble Desk.

During normal-hours the animal facility staff members monitor environmental conditions and place a service ticket with ORF for any problems noted. For emergencies, GSH will respond on-site within thirty minutes.

During after-hours for a Tier 1 alarm, maintenance staff will respond on-site within 60 minutes of the alarm; no notice is sent to animal facility management. For a Tier 2a alarm, maintenance staff will respond within 60 minutes, if not already on-site; the automated system will contact animal facility management if the room has been at the listed temperature for 2 hours and corrections have not occurred. For Tier 2 alarms, maintenance staff will respond within 60 minutes, if not already on-site; the automated system will contact the animal facility management immediately.

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]

Humidity Set Points and Alarm Limits[1]

Species	Set Point	Warning High Tier 1 Alarm Limit
Terrestrials	[3]	70%

Temperature Set Points and Alarm Limits[1]

Species	Critical Low Tier 2 Alarm Limit	Warning Low Tier 1 Alarm Limit	Set Point	Warning High Tier 1 Alarm Limit	Critical High Prolonged (2hr) Tier 2a Alarm Limit	Critical High Tier 2 Alarm Limit
Rodents	64°F (-8)	69°F (-3)	72°F –	75°F (+3)	76°F (+4)	78°F (+6)
Rabbits, and Ferrets	61°F (-4)	62°F (-3)	65°F –	68°F (+3)	70°F (+5)	72°F (+7)

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]

Temperature set points fall within the Guide's recommended dry-bulb macroenvironmental temperatures and are, consequently, set below the

[1] Non-standard set points and alarm ranges for individual rooms are only established with approval of the IC Animal Program Director and ORF.

[2] Room air temperatures are adjusted as needed to meet the water temperature requirements of the aquatic species being held. Sufficient air change rates are required to prevent condensation on room equipment and surfaces and maintain temperature requirements. More air changes than necessary cause increased evaporation of system water which increases the amount of replacement water added to the system and can decrease stability of the system.

[3] Typical facilities (with bulk humidification at typical AHU discharge air temperature, without room level humidification, and without dehumidification) provide limited humidity control. Expected room humidity ranges for specific room temperatures are below:

Temperature	65°F	72°F	76°F	80°F

thermoneutral zone to avoid heat stress. These resources and processes are provided to allow animals to control their temperature and avoid cold stress:

- Daily temperature fluctuations are kept to a minimum to avoid repeated large demands on the animals' metabolic and behavioral processes required to compensate for changes in the thermal environment.
- Rodents are socially housed, whenever possible or unless exempted by ASP, to support behavioral adaptation mechanisms.
- Rodents are normally housed on corncob bedding; however, nude mice are provided Tek-fresh (shredded paper) bedding but no nestlets (due to issues we have had with nestlets causing eye issues in nude mice).
- All mice (except nude mice and certain rats where the ASP approved exemption from enrichment for scientifically-justified reasons) are provided insulating nesting material or paper tubes unless exempted by ASP.
- Rodents are provided slotted cage floors to prevent animal wetting and evaporative chilling when housed within operant testing chambers.
- Primary enclosures are constructed from or contain materials with low heat conduction properties, e.g., polycarbonate rodent cages.

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.

HVAC system provides 10 or greater air exchanges per hour in all animal housing rooms.

- 100% fresh air is provided to the animal facilities after passing through a 30% efficiency pleated pre-filter and a 95% efficiency bag-type filter; air is removed from housing rooms through a 10% efficiency, disposable fiberglass media filter at the individual room level to the building exterior.
- Housing, testing, and most procedure rooms for animals are maintained under negative pressure gradients, and personnel areas are maintained under positive pressure gradient to service corridors. A minimum of 50 cfm airflow is used to maintain room air pressure differentials.

- Procedure rooms where surgery is performed are positive to the corridors.

Automated building systems provide continuous monitoring of supply and exhaust airflows, and control of supply boxes is yoked to trail exhaust boxes to maintain negative pressure gradients (or reciprocally for positive pressure gradients). Measurements are performed at least triennially by a commercial air-balancing vendor to verify the automated building systems' performance. In addition, as needed they perform room-level airflow smoke tests to assist in determining directionality of airflow.

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

Rodents are housed in microisolators on ventilated racks with each blower factory preset from 34 to 75 air changes per hour. Cage air flow is maintained positive to the room (except in BSL-2 rooms, where cages are negative in pressure relative to the room); all rooms are negative in pressure relative to the hallways.

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.

Ventilated racks supply individual rodent cages with HEPA filtered room air, which is a mixture of fresh room air and racking system exhaust.

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).

N/A

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).

N/A

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.

Exterior noise abatement

- Outdoor sources are muted due to below grade location of the Redacted by agreement

Interior noise abatement

Airborne transmission:

- Spaces for noise-generating mechanical HVAC systems, boilers, generators, bulk trash compactors are located on separate floors.
- Spaces for noise-generating cage wash operations are isolated by corridors and with heavy-grade, gypsum drywalls with sound- attenuating battens or concrete block walls, which extend to the concrete slab above.
- Husbandry and corridor operations noise sources are compartmentalized by:
- Automatically closing doors at cagewash.
- Sound-attenuating corridor doors with mechanical closers, door frames with rubber bumpers and elastomeric gaskets, and automatic drop door sill closures.
- Ventilated cage rack mounted blowers contain rubber isolation pads.
- Polyurethane or cushioned rubber casters are used on rolling stock, and rack casters are lubricated after cage wash sanitation to reduce corridor circulation noise.
- CMS staff has been advised of the problems that noise can have on laboratory animals and are instructed to keep noise to a minimum. Staff takes care in the handling of carts and racks, and rubber bumpers are fitted to some equipment. All doors in animal rooms and corridors are kept closed, and activities that produce noise are performed in separate areas whenever possible.
- Fire alarm annunciators and public address speakers are located within corridors, outside of housing and testing rooms to minimize potential animal disturbance; fire alarm drills are scheduled and coordinated through the animal program to avoid disruption of behavioral studies.
- Heavy-grade drywall or suspended ceiling panels cover an interstitial space containing wiring, utility distribution pipes, and HVAC ductwork to mitigate noise transmitted from the concrete slab of the floor immediately above the vivarium.
- Noise originating from building repair and maintenance work, e.g. drilling, demolition, renovating, is scheduled, coordinated through the animal program, and communicated to scientists to avoid disruption of sensitive animal studies.

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 and AWRs are the primary references used by the ACUC to verify adequacy of space provided for all research animals.

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]

Housing animals for more than 12 hours in a less-than-Guide-specified housing space require an ACUC exemption approval. Some behavioral testing systems provide less than required space, however, due to the short time animals are in these systems, these do not qualify as exemptions.

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).

Rabbits – privacy shelf, clear divider with contact holes
Guinea pigs – covered refuge
Nonhuman primates (we currently do not house or hold NHPs) – shelves/perches

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).

Mice – nestlets, paper tubes
Rats – Nyla bones
Rabbits – jingle balls, metal chains, bunny blocks, mirrors, dumbbells, caretaker interaction (grooming), rotating food enrichment
Guinea pigs – Nyla bones, rotating food enrichment
Nonhuman primates (we currently do not house or hold NHPs) – two floor toys and one hanging toy

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

i. Describe institutional expectations or strategies for social housing of animals.

The standard practice is that all rodents are group-housed, rabbits and nonhuman primates are socially housed.

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.

The ACUC has approved single housing for rodents in the case of some diet studies, exercise studies, and in the case of aggressive animals.

The ACUC has also approved single housing for rabbits. Some rabbits (in our experience, most males) are housed individually upon arrival due to the short duration on site and the potential negative effects on research should an injury occur. The CMS has developed transparent dividers with holes drilled in them to allow these specific rabbits to have visual and tactile access to neighboring conspecifics (see below). Exceptions to even this arrangement may include animals that may be stressed and cannot accommodate to each other when placed in these conditions. This is evidenced by their making aggressive actions towards each other, avoiding the shared side of the cage, or other symptoms indicating failure to socialize with each other even with a plastic divider. In these cases, the veterinary staff will observe and document these activities and the veterinarian may determine that these animals require permanent removal from social housing consideration. The veterinarian will in these cases provide that information to the ACUC for approval, or through the PI for amendment to their protocol for such removal. These animals will still remain in the same room, with olfactory and audible contact with each other, but without the visual and tactile contact that caused the stress.

Aged nonhuman primates may also be housed individually due to social incompatibility.

The ACUC has established a policy addressing the housing of social species.

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

Individually housed rabbits are in racks with clear polycarbonate separators. The separators are fabricated with multiple approximately 2-inch holes to allow contact between neighboring animals. Rabbits are provided with enrichment devices as well as regular interaction with the care staff.

Individually housed rodents are provided additional environmental enrichment that may include a combination of a nestlet, a paper tube and/or plastic enrichment manipulanda.

We do not currently house NHPs nor are expected to do so for 6-9 months from date of PD submission. Should NHPs be housed individually, for either scientific justification or because of inability to be safely housed with other NHPs, appropriate information will be documented in their records and animals will be provided additional enrichment in the form of manipulanda or other enrichment devices. Safe and positive interaction with the care staff, as well as being in the same room as conspecifics, will also occur to enrich their environment as much as possible.

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.

The ACUC evaluates the justification for exceptions to social housing for consistency with the scientific goals of animal use during review of initial submission and modifications of the Animal Study Proposal. Enrichment practices are reviewed semiannually during the ACUC animal program review. In addition, the ACUC has an established policy addressing the housing of social species. Enrichment SOPs are reviewed and updated at least triennially.

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.

Animals are allowed a 48-hour acclimation period prior to use. Husbandry procedures are standardized in a routine manner. As much as possible, consistency in personnel is maintained for longevity studies.

For experimental procedure habituation, ASPs describe training practices to acclimate animals to restraint devices and experimental procedures (see SECTION B.c.iii.2) (Program Oversight -- Special Considerations -- Physical Restraint). Such examples include handling rodents for multiple days prior to repeated blood collections and similar basic habituation procedures.

Behavioral testing: For both humane and scientific reasons we habituate animals to their behavioral testing modalities as much as possible prior to testing. All mice are allowed to habituate to arrival in the vivarium for at least one week prior to behavioral testing. Whenever possible we avoid testing mice on days of cage bedding change in order to minimize additional stress on the animals. Some tests require 7 or more days of testing, thus making this unavoidable. We always allow mice to habituate to the test room for 15-60 minutes prior to behavior testing. In some behavior tests we allow additional habituation to the testing chamber itself for 1-60 minutes prior to testing, in order to reduce stress at the time of data collection. These tests include odor exploration, buried food test, sociability, treadmill-based gait test, novel object recognition.

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).

N/A

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.).

N/A

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.

N/A

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).

N/A

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

N/A

iii. Describe how animals are captured.

N/A

C. Animal Facility Management

1. Husbandry

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

i. List type and source of food stuffs.

All feed, bedding and enrichment items, except for vitamin-C-enriched or lab-specific special diets, are autoclaved prior to use. Some diets arrive already irradiated.

Base diets provided [unless otherwise specified, diets are from Envigo]:

- 2018SX Global 18% Protein Extruded Rodent Diet (Sterilizable)
- 2019S Global 19% protein autoclavable rodent diet
- 2041 Guinea Pig Diet
- 2030 Global Rabbit Diet
- Hi/Low Salt Diets
- Caloric Restricted Diets – Envigo, Dyets, BioServ
- 5038 Primate Lab Diet (we currently do not house or handle NHPs)

Animal care and research programs supplement the basal diets with:

- Dough diet (Love Mash®, BioServ)
- HydroGel – ClearH2O
- NutraGel – BioServ
- TransGel – ClearH2O
- DietGel – ClearH2O
- NutriCal – NLS
- Supreme Mini Treats – BioServ
- F1011 Timothy cubes – BioServ
- Rabbit Stix – BioServ

- Bunny Blocks – BioServ
- Primate Prima-Treats (we currently do not house or handle NHPs) – BioServ
- Veggie Chips - BioServ

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

The majority of animal diets/supplements used are shipped directly from the manufacturer, Envigo and BioServ, to the Redacted by and stored in designated feed storage areas without any vendor interim warehouse involvement.

NIA does not use any bulk food storage facility.

Redacted by room Redacted by agreement is the designated food storage room. All food is stored in original bags on plastic pallets slightly away from the wall. Temperature is maintained at approximately 70°F. Vermin control is provided by NIH Integrated Pest Management Program Service. All doors have sweeps.

Rabbit, guinea pig, and nonhuman primate feed (we currently do not house or handle any NHPs) is kept in dedicated vermin-proof, lock-top Rubbermaid buckets, lined with plastic bags. Sanitation and shipping labels from each food bag are attached to each food barrel to verify the milling date of the food.

A small quantity of rodent feed is kept in a dated, autoclaved microisolator cage with filter top lid in each rodent housing room for investigator use during weekends, holidays, or after hours.

iii. Describe special food preparation areas, such as feed mills 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).

N/A

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

Mice and rats are fed through slotted, stainless steel feeders hanging inside the cage or wire bar feeders. Rabbits and guinea pigs are fed through J-feeders. All rodents and rabbits are fed *ad libitum*, with the exception of caloric restricted rodents, which are fed according to the animal use protocol. Nonhuman primates are fed a specified number of biscuits in a food hopper twice daily. This is enough to maintain appropriate body condition, unless they are on a feed restriction diet, in which case they are fed in accordance with the approved animal use protocol.

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

All feed, bedding and enrichment items, except for Vitamin-C-enriched or lab-specific special diets, are autoclaved prior to use. Some diets arrive already irradiated.

Animal feed is ordered on an as-needed basis, as determined by the Animal Facility Manager's weekly inventory. The reorder level leaves a small excess from the previous week's consumption on the next shipment's anticipated day of receipt; during the winter months, more feed is kept on hand in case of bad weather. As most of the food in stock is used prior to the delivery of the next shipment, rotation is not a problem.

- Upon receipt, bags are inspected for mill date, punctures, tears, vermin infestation, and wetness.
    - If defects are noted, the bag is returned immediately through the deliverer or excluded from stock storage for later return.
    - The mill date is verified and any bag that is more than 90 days past milling is rejected (rodent chow).
    - The mill date is verified and any bag that is more than 180 days past milling is rejected (NHP/GP/R).
    - Feedbags are placed with mill dates visible in front.
    - Feedbags are stored and used in a manner to ensure the oldest feed is used first (first in/first out).

Feed testing is done for our most commonly-used diets, the 2018SX (stock rodent diet) and 2019SX (breeder chow) by submission of samples as requested by the laboratory animal nutritionist at DVR.

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 [Redacted] is supplied water through the municipal water system managed by the City of Baltimore. Water is filtered by reverse osmosis and chlorinated 3.0 – 4.0 ppm for provision to animals in water bottles. ORF/GSH controls the chlorination process. Water for all species is provided via water bottles only.

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

Treated water is tested daily to confirm free chlorine (3.0-4.0 ppm) treatment at the level of the bottle filler manifold. Water is also periodically tested by the ORF / GSH.

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

N/A

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

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

All bedding is supplied by Envigo:

- Tek Board Pan Liners – standard pen liners for rabbit caging;
- 7092 1/8" corncob – standard direct bedding for stock rodent cages;
- 7093 Teklad Shredded Aspen Bedding – for breeding rodents;
- 7099 Tek Fresh Bedding – for heavy rats with foot pad issues and post-surgery animals

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

The majority of bedding used is shipped directly from the manufacturer, Envigo, to the NIA animal facility [Redacted] Room [Redacted by agreement] is the designated bedding storage room. Bedding is stored on plastic pallets away from walls. Temperature is maintained at approximately 70ºF.

Vermin control is provided by NIH Integrated Pest Management (IPM). There are door sweeps on all doors and IPM provides extremely detailed

weekly reports describing issues, potential sources of future issues, prevention strategies, and related information.

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

Bags are inspected upon receipt. Bags that are broken, patched, wet, or show signs of contamination are rejected or segregated for return.

Bags are opened and emptied into an automated bedding dispenser hopper located in the feed and bedding storage room. Bedding is visually examined upon opening for consistency of texture, dryness, and the presence of vermin and for freshness of aroma, and, if abnormal, segregated for review by the facility manager.

All feed, bedding and enrichment items, except for Vitamin-C-enriched or lab-specific special diets, are autoclaved prior to use. Some diets arrive already irradiated.

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.

The NIH Division of Veterinary Resources (DVR) provides transportation services between Redacted by agreement and other NIH programs on the Bethesda, Maryland and Redacted by agreement campuses. Transport is done in dedicated vehicles that have climatically controlled cargo compartments separate from the passenger compartment; the temperature of the compartment is independently controlled.

Animals are only rarely transported from the NIA to other locations using NIA vehicles. If required, the following NIA vehicles are used for animal transport: Any Government-owned vehicle that is temperature controlled may be used for rodent transport. A log book exists for each vehicle used, where temperatures and humidity are recorded at the beginning and end of transport and every 2 hours thereafter. Animals are put into clean, filter-top transport boxes. Vehicle sanitation occurs after transport in accordance with NIA/CMS SOPs.

All personnel driving vehicles have completed all required training for personnel having animal contact and are part of the Occupational Health surveillance program Appropriate temperatures, as controlled by manual AC/heating, are documented prior to transport.

Commercial animal transporters (e.g., World Courier) are sometimes used.

Vehicles appearing to not meet standards for transport of animals may be inspected by CMS personnel and may be rejected for transport. Similarly, incoming animals arriving in vehicles that do not appear to meet standards of contaminant or temperature control may be rejected by CMS.

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.).

The animal facilities use HEPA-filtered wet-dry vacuums, foam sprayers, and waste disposal units. These items typically remain in the same room and are manually or machine-sanitized prior to movement to another room or when visibly dirty.

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.

Rabbits (non-contact) – pans are changed at least weekly, or more frequently as needed.

Mice/Rats – (individually ventilated caging; contact bedding) – cages are changed every week, or more frequently as needed.

Guinea Pigs – (individually ventilated caging; contact bedding) – cages are changed at least three times per week, or more frequently as needed.

Nonhuman Primates (we do not currently house or hold NHPs) – (non-contact) – Pans are changed once daily, or more frequently, as needed.

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

N/A – there are no approved exceptions.

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

cages/enclosures.

Soiled non-hazardous bedding is removed in the cage cleaning room Redacted by agreement via the bedding waste vacuum disposal system.

Certain hazardous agents require bedding to be dumped and bagged within the room in a biological safety cabinet.

Clean bedding is placed into cages in room Redacted by agreement via the automatic bedding dispenser.

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.

N/A

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).

Tunnel and cage/rack washers – pressure and temperature gauges are checked daily. Temperature-sensitive monitoring tapes are used daily. The mechanical operation of the cage washers and their efficacy to sanitize objects is evaluated on a regular basis.

Visually: Water temperature readings on the machines' touch screens are visually verified at the start of each day's operations. A temperature recording label (TEMP DOT 180, Pharmacal) is run twice daily and attached to the log of results. Cages and racks are visually inspected for residual debris as they exit the washers.

Microbiologically: Hycheck contact slides are biological indicators used to perform random monthly quality assurance audits of caging and caging equipment post-autoclave. Rochester Midland Company (RMC) performs monthly quality assurance testing. Findings that

suggest inadequate sanitation are investigated and corrective actions are taken.

Autoclaves – vacuum pressure and cycle times are checked daily. Temperature-sensitive tapes are included with each load.

Scheduled preventative maintenance is provided to autoclaves by contract.

Sanitation and cleaning of behavioral and other sensitive experimental equipment located in animal use areas is performed by research staff; effectiveness is visually monitored by laboratory staff and CMS staff during regular walk-throughs of these spaces. Cleaning supplies and equipment are provided within each laboratory-managed procedure room.

b) Describe preventive maintenance programs for mechanical washers.

Avant-Garde Scientific, Inc. (Damascus, MD), a commercial biomedical equipment maintenance company, is contracted to perform repairs and quarterly preventative maintenance services to cage and rack washers. Avant-Garde also provides 24-hour emergency service for machine failures. All cagewash problems are repaired promptly.

Daily and weekly preventative maintenance for cage and rack washers is described in SOPs. Animal care staff:

- Clean sump screens/filters.
- Remove headers or pipe caps to manifold/arms.
- Clear jet orifices.
- Visually inspect door seal gaskets/curtains.
- Drain and flush wash and rinse tanks.
- Descale tanks and washer interior (weekly).
- Flush air or steam lines (weekly, as appropriate)

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.

Non-hazardous, soiled bedding and food are dumped in dirty side cagewash into a vacuum waste disposal system, which accumulates the

bedding in an outdoor dumpster for weekly removal by an outside contractor.

Biohazardous waste is autoclaved before being placed in the non-hazardous waste pipeline or it is dumped into Medical Pathological Waste (MPW) boxes for incineration by a contract service.

ii. Animal carcasses.

For experimental animals that may die unexpectedly, carcasses are placed in a plastic bag and refrigerated for forty-eight hours to permit investigators to retrieve animals or to request necropsy before transfer to the freezer.

Animal carcasses and tissues are collected into plastic bags, sealed, and placed into cardboard MPW boxes inside a dedicated carcass freezer in room Redacted by agreement When filled, MPW boxes are sealed and stored in the dedicated cold storage room Redacted by Room Redacted by agreement and later incinerated by the MPW contractor.

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).

NIH Integrated Pest Management (IPM) program provides pest control within the NIA animal facility. The basic concepts of the program focus on identification of pests within the facilities, elimination of entrance or harborage, education of the animal care staff on control methods, and, lastly, treatment with the minimum amount of pesticide required to eliminate pests.

Live traps with transparent tops are deployed by the contractor within the animal facility and monitored daily by the animal care staff for the presence of trapped research or feral rodents.

Logbooks are kept in the facility that contains details about the contractor's activities. Staff monitoring of traps is recorded on room log sheets.

They provide detailed weekly, monthly, quarterly, and semi-annual summary reports describing specific pests present, photos of any problem

areas, recommendations to resolve problems found, trends, and other valuable information that allows targeted measures for pest control.

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.

N/A

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

If use of pesticides inside the vivarium is required, animal users are provided at least one-week advance notification to give them time to consult with a CMS Veterinarian, if required, regarding potential impact to their studies, and allow time for evacuation of resident animals and/or adjustment of room differential pressures prior to any pesticide application.

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.

Regularly scheduled weekend and holiday care is provided by the animal care contractor, rotating regular staff. Staff performs health checks and treatments on weekends and holidays. The Facility or Regulatory Veterinarians are available 24/7, 365 days a year, via telephone, or in person if required, to provide emergency care.

Animal care staff is designated as Tier IB, Mission Critical, and are required to work during federal government closures (i.e. inclement weather) or other disaster/emergency situations.

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

N/A

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

Emergency contact phone numbers are conspicuously posted at facility entrances and throughout the animal facility.

Written instructions for veterinary and special husbandry care and directions for communication with veterinary, supervisory, research or facility' staff is provided to weekend/holiday staff. Individual Information for Emergency Treatment and Care forms (filled out as part of the ASP) are located within the appropriate animal housing room. The forms provide investigator contact information.

All problems encountered are brought to the Facility Manager's attention. Problems beyond the manager's control are immediately reported to the ARPM and the APD. If the problem is clinical and warrants veterinary care, the Facility or Regulatory Veterinarian is contacted.

In case of ventilation, heating/cooling equipment, or water system failure that threatens animal health, building engineers are available 24/7. The animal caretaker in charge contacts the facility manager, if the emergency requires animal care staff members to respond to the event.

Additionally, the NIA Safety, Security, and building engineering groups are provided a roster of emergency contacts.

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).

Mice and Rats: Ear tags and/or ear punches, and individual cage cards. Some experimental animals are identified by microchips at the request of the investigator.

Rabbits: Ear tattoos and cage cards.

Guinea Pigs: Ears marked with nontoxic marker and cage cards.

Nonhuman Primates: Tattoos and cage identifiers.

The CMS Progeny Database was created to provide a virtual real-time representation of the number of animals in any room at any given time. The database includes breeding animals, stock animals, experimental animals, non-weaned litters, and genotyping information. Every animal, regardless of species, has an individual entry in the database and a unique barcoded cage card.

This database generates uniform cage cards that include: ASP number, sex, strain, investigator name, contact information, source, genotype and date of birth.

b. Breeding, Genetics, and Nomenclature

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

Guidance and advice are provided at appropriate stages of use:

- Animal vendors and collaborating investigators provide genetic histories, and, as required, genotyping protocols to NIA investigators.
- The Regulatory Veterinarian and investigators provide guidance both during ASP preparation and during subsequent ACUC review.
- The veterinarians contact investigators when adverse phenotypes (overgrown teeth, micro-ophthalmia) arise in breeding colonies, advising against use of the animals in their breeding program.
- Contracted genotyping services are available to all investigators.

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.

The publishing investigator, reviewers, and editors are responsible for the correct nomenclature of the animals. The Animal Program Director and Regulatory Veterinarian are available to provide assistance.

Applicable guidelines such as those from the International Committee on Standardized Genetic Nomenclature for mice and the ILAR documents, "Standardized Nomenclature for Transgenic Animals" may be used to advise investigators of proper nomenclature.

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

Tissue samples are submitted to an outside genotyping laboratory for the majority of animals born at the Redacted by . Genotyping results are obtained electronically from the laboratory and automatically uploaded to the CMS Progeny database based on a unique animal identification number. The results for each animal are available for the investigators and veterinarians

to observe within the database. When an animal is removed from the colony, its record is archived but still available indefinitely for future reference. The database allows one to track back an animal's heritage including their genotype. Whether investigators do their own genotyping or use the genotyping contract, it is the investigator's responsibility to ensure the genetic authenticity of their breeding animals. CMS veterinarians are available to assist with genotyping questions.

Investigators are strongly encouraged to cryopreserve their unique strains.

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."

Principal Investigators describe anticipated signs of animal illness and disability in the Animal Study Proposal and propose humane endpoints and specialized husbandry practices.

Abnormalities noted by caretakers or technicians during work with animals are documented and managed using the system outlined in III,B,4,b. The Regulatory Veterinarian determines if a particular abnormality constitutes a phenotype that must be reported to the ACUC for inclusion in, and management through, the ASP.

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.).

The NIH DVR Animal Ordering and Contracts Unit procures rodents for NIA through established vendor contracts that define health status in terms of pathogens that are monitored.

- The vendors provide detailed descriptions of their disease monitoring and control programs to assure maintenance of acceptable health status, and NIH DVR monitors the quality assurance program of approved vendors.

- The contracts require each rodent vendor to immediately report any health status changes to the contract administrators, who in turn notify NIA of potentially contaminated past and pending shipments. Vendor health surveillance data is supplemented by the NIA in-house health surveillance program.

Import of animals from intramural, academic, and commercial sources not specified by the NIH contract must be approved by the NIA Rodent Import Officer.

- A health history of the source's animal facility is researched. Supportive documentation of the health surveillance program to include serological, parasitological, PCR or other appropriate evaluation, reflecting the current status of the exporting animal colony is reviewed. Information about colony size, location, husbandry techniques, special husbandry practices, genotype information is also requested. Requirements for further testing or prophylactic anthelmintic treatment are considered. If the animals are believed to be pathogen-free, importation is approved.

    - Imported rodents may be tested, while quarantined at the NIHAC or isolated in-house, to validate the source's disease-free status based on PCR/serological or parasitological test results before release to research or breeder colonies.
    - Requests to import rodents from sources with unacceptable health status are rejected, or rederivation, through an MOU for rederivation and cryopreservation services with the National Institute of Diabetes and Digestive and Kidney (NIDDK) or using a commercial company, is required. Rederived offspring are received upon review and approval of their health surveillance data.

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).

To the NIA: Animals are transported in dedicated climate-controlled vehicles in accordance with NIH Transportation policies and the Animal Welfare Regulations.

- Approved vendors deliver animals directly to the facility in vendor owned vehicles. Intramural sources deliver animals directly to the facility via NIH DVR operated, climate-controlled vehicles or via the NIA courier.
- Transportation of non-contract rodents between NIA and extramural institutions is portal to portal via commercial air carriers and local transport

after obtaining appropriate clearances. The Import/Export Coordinator is involved with shipments of animals from non-commercial sources.

From the NIA:

- The Import/Export Coordinator assists laboratory administrators to purchase shipping services through a commercial animal transporter - commonly (b)(4) The coordinator facilitates compliance with animal transportation requirements including provision of health certificates, obtaining sending/receiving institutions' addresses and husbandry and veterinary care contacts.
- The Import/Export Coordinator serves as shipping coordinator for packing up rodents for export in International Air Transport Association (IATA) Live Animal Regulations compliant disposable, plastic or cardboard, filter-protected shipping containers with sufficient internal food and water sources to maintain the animals until they reach their destination.

Additional animal biosecurity measures include:

- Animals are delivered to a dedicated animal facility loading dock.
- Shipping crates are transferred to a cart for transport directly to the animals' designated housing room. The exterior surfaces of the crates are spray misted with chlorine dioxide solution and then the animals are moved to the holding room for unpacking.

Within the NIA: Animals are transported to laboratories in their home cages or in vented cardboard transport containers by authorized animal users. Animals must be transported between floors using the freight elevator.

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.

Ongoing and episodic systems evaluate animals' health status for known or unknown infectious agents:

- The majority of animals are purchased on an NIH animal procurement umbrella contract that requires the vendors provide detailed descriptions of their disease monitoring and control programs to assure maintenance of acceptable health status prior to contract award. The NIH DVR:

monitors the quality assurance program of those vendors awarded a contract; conducts site visits of vendor facilities; requires vendors to notify the NIH within 24 hr of a confirmed positive result; and DVR pathologists periodically perform comprehensive necropsies and diagnostic testing on animals received directly from vendors.

- Daily animal observations by trained animal care technicians monitor for abnormal appearance and behavior.
- Diagnostic necropsies are performed on sick animals that are unneeded by researchers. Limited necropsies may be performed by the NIA veterinarians; comprehensive necropsies are performed by DVR pathologists.
- A sentinel surveillance program monitors for infectious agents:

  1) SD or F344 rats and Swiss Webster mice are purchased from a commercial vendor and are continuously used as sentinel animals for each rack in each centralized rat and mouse housing room. Sentinels are exposed to bedding used previously by other animals on that rack.
  2) Serology and parasitology are evaluated every three months from sentinels in each holding room.

- Pest control practices are applied building-wide, and live-traps are continually deployed and monitored to detect pest and vermin infestation within the animal facility. SOP directs elimination of escaped research and feral animals; risk-based testing of feral rodents is performed on any animal found loose within the animal facility.
- Imported non-commercial origin rodents are tested during entry quarantine or isolation.
- Biologic materials destined for administration into animals, identified in the ASP's Section L, Biological Material/Animal Products, may undergo PCR or Mouse Antibody Production (MAP)/Rat Antibody Production (RAP) testing to ensure the products are free from zoonotic and other rodent agents.
- Rabbit physicals are performed on arrival and quarterly thereafter.
- Guinea pig physicals are performed on arrival and then quarterly.
- Regular health monitoring and testing for NHPs is performed on the Redacted by agreement campus at the DVR central facility.

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

Procedures used to control entry of agents into the facility, or spread of agents within the vivarium, include:

- Only animals of a desired health status enter general holding rooms. NIA maintains a list of acceptable agents. Animals positive for agents that are not on the list are denied entry.
- Cage wash provides clearly defined areas for clean and soiled equipment and operations.
- The breeding barrier accepts only vendor animals. It is located beside clean cage wash and sterile caging moves directly from the autoclaves into barrier storage.
- Assignment of animal care personnel, traffic control patterns, and physical segregation diminishes risks between populations (e.g. breeder colony and experimental rodent housing rooms) within defined areas: barrier, conventional housing, and quarantine.
- Rooms are equipped with programmable electronic card readers and investigator animal holding is consolidated to single rooms whenever possible.
- Use of dedicated PPE, ventilated caging systems and micro-isolator changing practices within laminar flow change stations.
- Transport of rodents within microisolator cages or filtered boxes.
- With the exception of animals undergoing MRI, animals that exit the vivarium are not permitted re-entry; they must be used in terminal procedures on the same day of exit.
- Room air pressure differentials aid in agent containment.
- Extensive practices and procedures employed, ensure sanitation of supplies, equipment, and the facility to reduce the potential for cross-contamination:
- Disinfectant (Clidox-S) is regularly applied to disposable gloves when changing cages, the exterior of shipping crates, cart wheels, etc.)
- Animal care staff is precluded from owning pet rodents which may serve as a source for contamination; research staff is queried and educated during orientation about risks from pet rodents and from contact with animals at collaborating institutions' animal facilities. Building management staff is additionally briefed on contamination risks from vermin and feral pests and from non-biosecure building areas, e.g. exterior dumpster.
- Visitors must be approved by the APD and are queried about contact with rodents. If there has been contact in the preceding 24 hours, visitors may be required to wear a full Tyvek instead of a lab coat, or shower. If the risk of contact with infected rodents is deemed to be high, the visitor will be refused access.

If a positive result is found in resident animals, the room or rack is quarantined based on the likelihood that the finding is a true positive. Once a positive is confirmed, an outbreak investigation and eradication plan is formulated. The aim of the outbreak investigation is to determine the location of all infected

animals within the positive area plus the potential of spread to other rooms and facilities. Information collected during the outbreak investigation is used to identify and correct deficiencies that led to the outbreak. Generally, a test and cull program are initiated; in some cases, animals are treated. Investigators are notified about the outbreak with phone calls, e-mails, and at Lab Chief, ACUC and Advisory Committee to the Animal Program (ACAP) meetings. Discussions about positive results and plans to contain and eradicate an agent may also occur at CMS led meetings with investigative staff.

The method and timeline for elimination of the infectious agent depends upon the agent, population of animals infected, and the likelihood of spread and purpose of the study.

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

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

Mice and Rats: Although newly received rodents from non-approved vendors or non-commercial sources are segregated before release to the general population, animal caretakers evaluate rodents during initial receipt.

- The rodent shipping label (e.g. investigator, strain, sex, number, and source) is verified with the log of anticipated deliveries.
- The animals are transferred to the assigned housing room.
    - Exterior surfaces of rodent shipping crates are sprayed with disinfectant (Virkon-S) before unpacking, and rodents are examined for overt signs of disease, placed into cages, and identified by cage card.
    - The facility manager, veterinary technician, and/or veterinarian are immediately notified of abnormal animals.

Rabbits: *Rabbits are only received from approved vendors*. The animal care staff inspects each rabbit as it is received; any obviously sick or injured animals are examined and treated by the veterinarian. After a minimum 24-hour stabilization period, rabbits undergo a health assessment that includes a physical examination by a veterinarian. At that time, an individual medical record is created.

Guinea Pigs: *Guinea pigs are only received from approved vendors*. The animal care staff inspects each guinea pig as it is received; any obviously sick or injured animals are examined and treated by the veterinarian. After a minimum 24-hour stabilization period, guinea pigs undergo a health assessment that includes a physical examination by a veterinarian. At that time, an individual medical record is created.

Nonhuman Primates *[we do not currently house or hold NHPs]:* Prior to transport from Redacted by agreement the investigator provides the veterinarian with a health history of each animal that includes all conditions requiring medical treatment. An ample supply of all medicines and food (including environmental enrichment) are shipped with the animals. The facility veterinary conducts a visual cage side evaluation upon arrival from the Redacted by agreement At that time, an individual medical record is created.

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.

Random source animals are not used. Only animals that were bred and raised specifically for research are used.

- Health status evaluation, of purpose bred rodents and rabbits from approved sources, is limited to initial animal receipt and evaluation; hence, they are placed directly into research and breeding colony housing rooms.
- Rodents originating from non-approved vendors and or non-commercial sources, determined by the Regulatory Veterinarian to be low risk, are housed in the isolation room Redacted by building, room Redacted by agreement and are released to animal housing rooms based on fecal and fur PCR screening results.
    - Pooled PCR panels are run on all animals from a shipment
    - Animals are placed on Fenbendazole-medicated feed upon arrival and spot-treated with selamectin.
- High-risk imports are shipped to the NIA Rodent Quarantine Station, Redacted by agreement for quarantine, testing, prophylactic anthelmintic treatment for endoparasites (fenbendazole medicated rodent food) and ectoparasites (topical selamectin).
- If animals test positive for pathogens that would preclude entry into Redacted by agreement research and breeding colonies, they can be rederived commercially or under an MOU that NIA maintains with NIDDK, treated, held for passive elimination of disease followed by re-testing , or euthanized.
- Redacted by agreement provides quarantine services for all non-human primates obtained by any NIH institute.

Rodent isolation is performed in Redacted by agreemen Room Redacted by agreement, a dedicated room with a negative differential pressure to adjacent areas. The isolation room is located away from other animal housing. It does not meet all NIH criteria to be officially designated as an NIH "isolation room", however, it is sufficient to meet industry

requirements to appropriately separate incoming animals that are at a certain known pathogen status until further testing is completed. It contains one Nuair Class A Type II biosafety cabinet.

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

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.

Redacted by
Room is used for breeding mice and only accepts vendor animals. The animal facility is roughly divided into mouse and rat holding and procedure rooms. Infectious organisms, infectious to multiple species, are not allowed to be present in any specie e.g. pneumonia virus of mice is excluded from rats, mice and guinea pigs. The only location that might result in mixing of animals of different health status, is the import isolation room. While all imports are closely vetted, importation of animals carries some infectious risk. Risk is being balanced against monetary costs of quarantine in this case.

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

Rats and small numbers of guinea pigs are housed in the same room Redacted by agreement Also, if necessary, rats and mice may be housed in the same room designated for imports Redacted by agreement if shipments overlap in time.

c. Describe isolation procedures and related facilities for animals.

If illness appears to be potentially hazardous to other animals, an animal can be removed to an individual cage and/or to an unoccupied animal room until sent to pathology or euthanized. If large numbers of animals become infected with an excluded pathogen, rooms are quarantined until an investigation of extent is performed and an eradication plan decided. Research-critical animals can be moved to building Redacted by agreement on the main NIH Bethesda campus. The facility is operated by the NIH DVR.

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.

Animal care staff, who are trained to recognize signs of disease and abnormal behavior of the animals they work with, make health observations twice daily, Monday through Friday, and once daily on weekends and holidays. They report ill and other abnormal animals to the veterinary care staff for timely examination, triage, and treatment.

- The veterinarian and facility management provide animal caretakers with on-the-job training during staff meetings or training sessions, AAALAS certification training, and while performing their assigned duties.
- Animal caretakers report abnormal observations on the animal monitor sick report generated in the Progeny database. If pain or distress is present, the animal caretaker, weekend supervisor, or facility manager, also verbally notifies the facility veterinarian or veterinary technician immediately.
- Investigative staff are encouraged to report abnormalities they notice to the animal care staff, managers, or veterinarians.
- **After hours**, including weekends and holidays, the animal care and research staff may contact the veterinarians and facility management through personal electronic communicators (mobile phones). The animal care staff additionally contacts the on-call veterinarians or veterinary technician to discuss triage and treatment of newly emerging or previously identified abnormal animals.
- Animals that are found dead are electronically reported through the Progeny database to the veterinary and appropriate research staff.

Many of the animals involved in behavioral research are also observed daily during experimental testing sessions and within procedure areas by researchers, who are able to note subtle behavioral or body weight changes. Methods of communication between research staff and animal/veterinary care staff is described directly below, section III.C.1.b.

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

Research Staff:

- As described above in section II.C.1.h.ii., posted emergency signage provides research and program support staff with contact information for responsible animal care and/or veterinary personnel.
- The research staff communicates to the animal/veterinary care staff directly by telephone or electronic mail, by verbally informing the animal caretaker, or by submitting an animal health report through the Progeny database.

Animal/Veterinary Care Staff:

- The animal care staff communicates animal health issues to veterinary care staff via a daily electronic health report, and, verbal notification for animals in pain or distress via telephone or electronic mail.
- The animal care staff also directly notifies research staff of animal mortality through the Progeny database.
- The veterinary care staff communicates animal health issues to researchers through the Progeny database, including the progress of on-going treatments, and inquiries of the investigators' plans for the disposition of the affected animal through electronic mail, and they communicate verbally by telephone to clarify recent research use and, for moribund animals, to rapidly schedule euthanasia.
- Emergency contact information for Principal Investigators, or responsible designees, for both during and after work hours, is collected for each ASP and maintained electronically. These Instructions for Emergency Treatment and Care sheets are laminated and available in the appropriate housing room(s) for each ASP.

For complex or recurrent health concerns, investigators, veterinarians and caretaker staff may meet.

After hours, including weekends and holidays, animal caretakers and research staff can contact the veterinarians, veterinary technician, or facility managers by personal cell phone or email. Contact information is posted at the facility entrance and at several locations within the facility.

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.

A majority of the animals housed in the 

are purchased under a DVR held procurement contract that requires vendors to maintain a strict surveillance program and rapidly report confirmed outbreaks in their facilities. When a vendor reports an outbreak, DVR contacts all investigators who received animals from that vendor during the period of concern.

Rodents: Daily animal observations by trained animal care technicians, submission of sick animals to veterinary pathologists, and a sentinel surveillance program monitor the health of rats and mice.

SD and/or F344 rats and Swiss Webster mice are purchased from a commercial vendor and are continuously used as sentinel animals for each rack in each rat and mouse housing room.

- Each rat or mouse housing rack holds a single sentinel cage containing 1-2 SD or F344 rats or 2 Swiss Webster mice, respectively.
- Sentinels are exposed to bedding used previously by other animals on that rack. During scheduled cage changes, soiled bedding samples are pooled from one side of each rack and used to bed the sentinel's cage on that side.
- Sentinel rodents are either sampled or submitted to the diagnostic laboratory on a quarterly basis. Serum, fecal and tape test samples are submitted three times a year and animals are submitted once a year according to SOP Attachment 3411B. Organs are grossly examined for lesions; DVR pathologists perform histopathology on any tissues with gross lesions. The pelage and cecum are removed for direct visual examination to detect, respectively, ectoparasites and pinworms.

After receipt of test results for submitted animals (every 6 months), the remaining sentinels are culled and new sentinel animals are placed.

Rabbits: Physical examinations are performed upon arrival and on a quarterly basis thereafter; however, it is rare for a research rabbit to be in the facility for a month. Rabbits are given limited rations for the first three days after arrival to curtail incidents of enteropathies. Rabbits are single-housed in environmentally enriched cages with water bottle access. Rabbits have individual medical records.

Guinea Pigs: Physical examinations are performed upon arrival and on a quarterly basis thereafter.

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.

Veterinary care is continuously available: Contact information is posted in the animal facility to reach the APD, facility veterinarian, backup emergency

veterinarian, and veterinary technician. In addition to animal care personnel on site, a facility manager is on-call for each weekend and holiday.

Procedures for animal care and research staff to make timely reports of animal injury, illness, or death during and outside of regularly scheduled hours is described above in Sections II.C.1.h.i. and III.C.1.a..

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

The APD, acting as the Attending Veterinarian, delegates the authority to the on-call veterinarian and backup emergency veterinarian to provide emergency care including treatment for illness and pain, suspension from use in a study, and, when necessary, euthanasia. Although the veterinarians attempt to accommodate both scientific as well as animal welfare objectives, e.g. rapidly schedule terminal perfusion or collect tissues after euthanasia, the veterinarians have the authority to appropriately treat any animal in a timely manner to relieve potential pain or distress.

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.

Medical records for individual rabbits, individual guinea pigs, individual monkeys, individually identified rodents that are currently receiving treatment or being monitored, and postsurgical rodents are maintained.

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.

Rabbit medical records are maintained by the Veterinary staff in the holding room, Redacted by agreement in a 3-ring binder. Rodent treatment sheets are maintained in each animal holding room. Each PI's ASP identifies the researcher(s) responsible for documenting postsurgical rodent observations. The veterinarians (including the Attending Veterinarian), veterinary technician, and ACUC have access to medical records; records are made available to investigators, if needed.

Permanent, written rabbit medical records are created upon arrival. Written entries are added to the medical record to summarize ancillary findings.

All rodent cases are entered into the Progeny database system which can be sorted by various means (room, strain, investigator, condition etc.). The database generates an animal health report, which is sent by electronic mail to researchers. Researchers are informed of progress and prognosis through verbal communication and electronic mail.

Postsurgical records identify the animal, date and type of surgery, and documents provision of ASP specified treatments, e.g. postoperative analgesia, and or observations of animal activity and surgical wounds.

[We currently do not hold or house any NHPs]. Historical medical records for NHPs sent to NIA for imaging are available electronically through DVMax (a medical records database), which is maintained by the DVR. A paper copy medical record is created when a monkey arrives at the NIA. Following the MRI, a copy of the records generated at NIA are sent to Redacted by agreement with the monkeys and uploaded to DVMax.

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

The Attending Veterinarian:

- Delegates authority for oversight/management of record-keeping to the Facility Veterinarian (Clinical Veterinarian), who ensures complete and accurate records and compliance with application policies and guidance.

- Delegates authority for review/quality assurance of records to the Veterinarian for Regulatory and Policy Compliance (aka Regulatory Veterinarian).

Both the Clinical and Regulatory Veterinarian may create or add to animal records during the performance of clinical duties.

The Regulatory Veterinarian is also the ACUC Attending Veterinarian and, as such, also provides review of records as required during semiannual facility inspections or as otherwise requested.

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

a. In-house diagnostic laboratory capabilities.

An in-house laboratory is available to detect the presence of endo- and ecto-parasites. The lab is also used for gross pathology exams and sample preparation.

Within the NIH, the intramural DVR's diagnostic laboratories perform environmental and clinical bacteriology, parasitology, diagnostic PCR testing, gross necropsy, and histopathological services by board-certified veterinary pathologists.

b. Commercially provided diagnostic laboratory services.

The NIH DVR diagnostic laboratories contract with IDEXX to perform rodent serological and PCR testing.

c. Necropsy facilities and histopathology capabilities.

A dedicated, common use necropsy area is located in Redacted by room Redacted by agreement which contains two downdraft stainless steel necropsy tables with sinks, euthanasia equipment with wall mounted CO2 cylinders, an isoflurane anesthesia machine set-up, and a carcass freezer.

Rooms Redacted by agreement and Redacted by agreement are the locations for CO2 euthanasia of rodents, commonly performed by the animal care staff; research technicians also perform ASP specified euthanasia of rodents followed by perfusion or tissue harvest in room Redacted by agreement CO2 euthanasia instructions are posted to supplement user training.

d. Radiology and other imaging capabilities.

N/A

5. Drug Storage and Control

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

The handling and safeguarding of controlled substances (including DEA regulated chemicals) from acquisition to disposal at the NIH are described in NIH Policy Manual 1345 "Handling and Safeguarding of Controlled Substances for Nonhuman Use" (PM-1345). While NIA maintains a Drug Enforcement Agency (DEA) certificate with a Baltimore address and is exempted from PM-1345, NIH requires the NIA to use a procedure similar to PM-1345 when handling a Controlled Substance (CS).

Controlled substances are maintained in room Redacted by agreement

Non-controlled drugs are ordered from commercial vendors and received by the ordering veterinarian or investigator.

- A supply of therapeutic veterinary drugs is stored in cabinets in animal treatment areas, and in rooms [Redacted by agreement], for use and dispensing by the Veterinary Care staff.
- The expiration dates of drugs and time-limited supplies are highlighted or marked to facilitate monthly review of stock.
- Each item is stored and used in a first-in-first-out manner.

b. Describe record keeping procedures for controlled substances.

All controlled substances are ordered, received, recorded on inventory, and stored according to NIH Policy Manual 1345, "Handling and Safeguarding of Controlled Substances for Nonhuman Use." The CMS also has a standard operating procedure that describes this process in detail.

The controlled substance custodian & program coordinator conduct regular audits of recipients' inventory to reconcile inventory with usage records.

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.

In the ASP, the PI identifies:

- Surgeon(s) and responsible postoperative care provider(s), and their training and experience in both the specific species and the specific procedures to be performed
- Location for the surgical procedure

Describes:

- Preoperative preparation,
- Anesthetic and analgesic use
- Surgical procedure method,
- Postoperative care, health monitoring and record keeping

Assures:

- Personnel performing surgical procedures are qualified through training and experience, *or that*:

- Personnel will be trained to perform the surgical procedures described in the ASP prior to any work with animals.

To ensure adequate pre-surgical planning, the following guidance is available to the PI:

- Species-specific Standard Operating Procedure and/or Appendix documents describe preoperative and postoperative procedures.
- The APD, Regulatory Veterinarian, or Facility Veterinarian can provide veterinary guidance for selecting analgesic and anesthetic agents, locating equipment and supplies, review of potentially painful procedures, potential outcomes and how to address associated pain or distress.

Investigators are encouraged to write training animals into their ASP or practice on training animals from the CMS training protocol prior to working on their own experimental animals. CMS veterinary staff is available to work with investigators to do a dry run of a surgery, observe a surgical technique or assist with any other surgical preparations.

- The ACUC Coordinator verifies training and experience records prior to ASP or amendment approval for surgery.

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.

Redacted by agreement rooms Redacted by agreement Redacted by agreement Redacted by agreement are used for survival and non-survival rodent surgery.

None of the below rooms are used as dedicated surgery suites nor are they equipped as such. All rooms may be used for rodent (major or minor) or rabbit (minor) surgical procedures. All rooms contain sanitizable surfaces in the area within and immediately surrounding the surgery locations. Surgery locations are in areas where traffic can be minimized and where there are no air vents blowing directly into the surgical space.

Each room has a sink, where the surgeon can wash hands prior to surgery and where gross debris can be removed from surgical instruments as required.

Portable surgical lights or room lighting are used for these surgeries.

By room:

- Redacted by agreement share a portable gas anesthesia machine and glass bead instrument sterilizer for rodents.
- Redacted by agreement two portable gas anesthesia machines.
- two gas anesthesia machines.
- two gas anesthesia machines.
- – gas anesthesia machine.
- two gas anesthesia machines
- three gas anesthesia machines.
- gas anesthesia machine

Ancillary equipment: Rooms used for surgery may also have portable stereotaxic devices, glass bead sterilizers, or a tabletop autoclave. However, these items can be moved so are not listed by-room.

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).

Survival surgery within the NIA facility has historically only been performed on rodents. While there is an approved minor survival procedure (subcutaneous telemetry implant in rabbits), this work has not yet started as of submission of this Program Description.

A major survival procedure is defined as any survival surgery that penetrates and exposes a body cavity or is capable of producing substantial impairment of physical or physiological functions.

The NIA ACUC defines "capable of producing substantial impairment of physiologic functions" broadly, to include the creation of diabetes models via subcutaneous implantation of pumps.

These procedures require preparation of the surgical field, patient and surgeon. Sterile gloves, equipment, and supplies are maintained during the operative procedures to reduce the introduction of infectious agents.

b. How is non-survival surgery defined?

Non-survival surgery is a surgical procedure performed on an animal that is euthanized before recovery from anesthesia.

Minimum requirements for non-survival surgery include clipping of the surgical site, cleaning of the surgical site, and that the surgeon wears gloves, as per The Guide. However, NIA surgeons will typically wear all survival-surgery required PPE (facemask, etc.), except for sterile gloves, because that is also the PPE required for handling animal tissues.

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

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

After induction of anesthesia, hair is removed from the surgery site with clippers or depilatory (as per SOP), the animal is positioned for surgery, and the skin incision site is disinfected by scrubbing with (iodophor or chlorhexadine-based) surgical soap followed by alcohol or sterile water rinse, repeated three times in an inside-out spiral pattern. For very small areas, one-directional scrubbing may be used, being careful to not drag the scrub implement over any hair outside the shaved area. Sterilized surgical instruments are used.

The surgeon dons surgical mask; hair cover; clean, disposable laboratory jacket; and sterile gloves. Non-sterile gloves may be used for non-survival rodent surgeries.

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.

Surgical instruments are steam sterilized by bulk or tabletop autoclaves in the CMS area or in investigator's tabletop laboratory autoclaves.

Effectiveness is confirmed by the use of temperature sensitive tape for the outer packet and/or a biological indicator inside the pack.

When used for sequential rodent surgeries, instruments may be disinfected and instrument tips dry heat sterilized in a hot glass bead sterilizer before and between rodent surgeries

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

Glass bead sterilization is an approved method of sterilization when multiple rodent surgeries are performed. Surgeons will start off with a sterilized pack and then use the glass bead sterilizer for subsequent surgeries.

Once items are placed in the bead sterilizer, only the tips are thenceforth considered sterile, and the surgeon's hands touching the handles are also no longer considered sterile – meaning that touching of the animal's surgical site with the hands can no longer occur.

d. Indicate how effectiveness of sterilization is monitored.

Effectiveness is confirmed by the use of temperature sensitive tape for the outer packet and/or a biological indicator inside the pack.

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

In general, the Principal Investigator provides technical surgical support. However, the veterinary staff is available for consultation as requested. We can also provide aseptic technique guidance, anesthesia machine use and anesthetic monitoring training, among other topics, either at the NIA or through the NIH.

CMS may assist with obtaining isoflurane machines and assist with machine setup, as required. CMS coordinates annual certification of all gas anesthetic machines.

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.

Anesthetic monitoring in mice and rats is accomplished by observation of respiration and evaluation of pain reflexes, typically the rear toe-pinch reflex. Research personnel monitor anesthesia of rodents during surgery.

The adequacy of anesthesia for acute, non-survival rodent procedures involving surgery, i.e. terminal perfusions, is monitored as described above for mice and rats. The adequacy of anesthesia for chronic, non-survival rodent procedures involving surgery, e.g. catheterization followed by imaging, is evaluated by

monitoring vital signs with electronic equipment. Measurement of heart rate, respiratory rate, oxygen saturation, rectal temperature, direct blood pressure, and/or arterial blood gas oxygenation may be recorded at intervals and the record maintained by the PI.

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.

NIA staff currently only perform surgery on mice and rats. While a rabbit telemetry implant amendment has been approved, work has not started as of submission of this Program Description.

Rodents recover from surgery in a clean cage with dry contact bedding, and are monitored until they are, as per NIH/ARAC Rodent Surgery Guidelines, "capable of purposeful movement".

- Designated research staff, typically the surgeon, provides primary postsurgical monitoring and care for at least three days. Animals must continue to be observed throughout the early postsurgical period until they appear to have normal appearance/appetite/feces & urine production and the incision is healing uneventfully.
- The date and type of surgical procedure is noted on a Special Observations card placed on the cage. The surgeon or research technician lists approved analgesic and therapeutic treatment on the back of the card. The animal care staff monitors postsurgical rodents as part of their twice-daily animal morbidity and mortality observations. The veterinary technician and veterinarian observe these cages weekly or more frequently as needed.
- Observed post-operative complications, including pain and distress, are treated, as per ASP, in consultation with a veterinarian.

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

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

The PI categorizes the level of pain and distress of animal procedures in the ASP application (Appendix 9) for all animals. During the review process, the ACUC and the designated AV evaluate categorization based upon the Principal Investigator's description, the ACUC's knowledge of the procedures involved, the NIH ARAC's Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain & Distress Categories, and principle IV of the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

The ARAC Guideline, "Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation" and species-specific standard operating procedures provide guidance to staff on pain and distress assessment through evaluation of changes in food or water intake, activity level, including species-specific behaviors, and surgical site complications that are suggestive of discomfort. Veterinary staff is familiar with published body condition scoring and grimace scales.

- The actual level of post-procedural pain and/or effectiveness of relief is assessed daily by animal and veterinary care staff during routine animal health observations and by research staff during close observation through execution of ASP defined, post-surgical monitoring practices.
- Postoperative observations are recorded on Special Observations cards and available for review by the veterinary staff for all species.
- The ACUC also assesses post-procedural pain in animals by observing animals during the semiannual inspection of the animal facilities.

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

- Required NIA training: All animal care staff are required to take AALAS Learning Library courses. All staff must complete in-person orientation and handling classes prior to being given facility access, during which pain and distress are discussed.
- Specific training for animal care/veterinary staff: the animal care and veterinary staff (all contracted) are provided additional pain and distress training mandated by their company and accomplished through online training that includes pictures and scenarios.

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.

The following pharmacologic agents are being used:

Rodents: buprenorphine HCL, buprenorphine SR, lidocaine, paracaine/tetracaine, ketamine, xylazine, pentobarbital, isoflurane, ibuprofen, acetaminophen, or meloxicam.

Rabbits: buprenorphine, ketamine, xylazine, pentobarbital, acepromazine, isoflurane, ketoprofen, flunixin meglumine, or meloxicam.

NHPs (we currently do not house or otherwise use NHPs, and do not plan on doing so for the next 6-9 months): ketamine, diazepam, medetomidine, isoflurane, buprenorphine HCL, meloxicam.

Non-pharmacologic means are employed to diminish fear and anxiety from procedures that can result in transient, mild discomfort, e.g. injection, as well as more pain, e.g. surgery.

- Many rodents are directly handled by research staff on a daily basis, at times for weeks, before starting an experiment to reduce animal distress which can confound sensitive operant behavioral studies.
- Surgeons position animals in a manner that avoids unnatural or unnecessary pressure or stress on body surfaces and joints, and handle and manipulate tissues in a manner that minimizes tissue trauma.
- Rodents are commonly housed individually during anesthetic recovery in clean, dry bedded cages to prevent damage to implanted materials and to prevent animal injury from another potentially painful or distressed, postsurgical rodent.
- Supplemental environmental warmth and warmed, parenteral fluids may be provided perioperatively to reduce physiologic stress and potential enhancement of distress during recovery from anesthesia and surgery. Heat and cold treatment modalities (e.g., hot water bottle) may be used alone or as an adjunct to analgesic treatment).

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

PIs must consult with a veterinarian regarding control of pain and distress potentially resulting from any ASP procedures.

Guidance for the application of anesthetics, analgesics and other pain control agents is provided in other forms, as well. Before any new agent is used, it is first discussed and approved by ACUC during ASP review. All CMS veterinarians (APD, CV and AV) are available for consultation, should problems or questions arise at any time before, during or after protocol submission. In addition, approved anesthetics for each species used are listed within CMS SOPs.

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.

Only rodent surgeries are done at the NIA at this time. While a brief (less than 10 min) subcutaneous telemetry implant surgery in rabbits was recently approved by the ACUC, work has not started as of submission of this PD.

The PI and research staff are responsible for monitoring the use of anesthesia and analgesia during surgery. During rodent surgeries the surgeon or other lab staff are responsible for animal monitoring. Lab staff may receive training on these topics from NIH resources or through in-lab training. Upon request, the CMS will provide training or support.

Post-surgical monitoring at the cage level for indicators of pain and distress is performed by both investigators and the CMS contract staff.

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.

N/A

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

- The Animal Program sponsors and coordinates an annual anesthetic vaporizer calibration certification, and the ACUC inspects vaporizers' date of inspection labels during their semi-annual facilities' inspections.
- The DOHS monitors the effectiveness of devices for scavenging volatile anesthetics including use of fume hoods and down-draft tables, local exhaust ventilation (snorkel) devices, and adsorption (f/air) canisters.

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.

The ACUC's policy on euthanasia is based on the following references, as applied to the species, age and condition of the animal:

- AVMA *Guidelines on Euthanasia*
- NIH ARAC Guidelines:
  - Guidelines for the Euthanasia of Rodent Feti and Neonates
  - Guidelines for Euthanasia of Rodents Using Carbon Dioxide

The ACUC may approve waivers from anesthesia use for physical methods based on scientific justification, provided by the PI in the ASP.

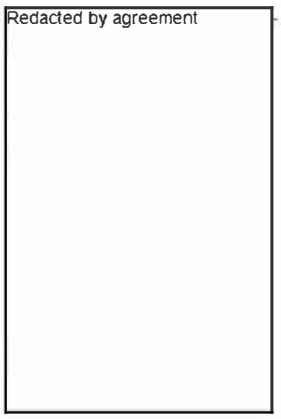
A tabulated description of ACUC approved methods is:

Mice
Cervical dislocation
Anesthetic overdose of barbiturate, isoflurane, xylazine/ketamine followed by decapitation, or thoracotomy with perfusion.
Decapitation
CO2 overdose followed by cervical dislocation (adult mice), decapitation (neonatal mice) or thoracotomy.
Fetuses – decapitation under residual CO2 or anesthesia of dam
Exsanguination under anesthesia (perfusion fixation)
Rats
CO2 overdose followed by cervical dislocation (neonatal rats), decapitation (<200 g) or thoracotomy.
Cervical dislocation for rats < 200 g
Decapitation
Exsanguination under anesthesia (perfusion fixation)
Fetuses – decapitation under residual CO2 or anesthesia of dam
Anesthetic overdose of barbiturate, isoflurane, xylazine/ketamine followed by decapitation or thoracotomy with perfusion.
Rabbits/Guinea Pigs/Nonhuman Primates
Barbiturate overdose, death is ensured by cessation of heartbeat or thoracotomy.

Rodent euthanasia may be performed in the Redacted by agreement animal facility necropsy room Redacted by agreement or in Redacted by agreement using carbon dioxide tanks with pressure/flow regulator, timer, and chamber lid. Posted, illustrated instructions regarding adjusting gas flow rates and dual-event timer are used for training animal care and research staff to ensure consistent and adequate duration of exposure to slow and rapid gas flows.

An isoflurane anesthetic machine is available within the necropsy suite. In addition, the necropsy room provides downdraft stainless steel necropsy tables with perfusate collection containers. Alternately, animals may be euthanized in these approved investigator laboratories:

- Redacted by agreement
-
-
-



Euthanasia locations are visited and evaluated at least annually during the ACUC semiannual facility inspections.

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

The ACUC checks euthanasia equipment during semiannual inspection of animal areas and the Regulatory Veterinarian inspects investigators' guillotines every six months to assess sharpness and, when needed, to coordinate sharpening by a commercial vendor.

The animal care staff is trained to review the pressure gauge on the carbon dioxide tanks to change and or reorder tanks before depletion. Posted, illustrated instructions regarding adjusting gas flow rates and dual-event timer are used for training animal care and research staff to ensure consistent and adequate duration of exposure to slow and rapid gas flows.

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

Death of an animal is confirmed by a combination of the following: absence of response to pain reflexes, absence of palpable heartbeat, prolonged cessation of breathing with cyanosis of tissues, and glazed eyes.

In addition, a secondary physical method of euthanasia is performed after CO2 or anesthetic overdose. For example, following CO2 euthanasia, NIA staff may perform cervical dislocation on adult mice, cervical decapitation (scissors) on neonatal mice, and thoracotomy on mice and rats. In other cases, such as after isoflurane overdose to apparent death, decapitation, puncture of diaphragm, or other methods may be used to guarantee death.

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.

The NIH [Redacted by agreement] building of approximately [Redacted by agreement] that was designed for use by the National Institute on Aging (NIA) and the National Institute on Drug Abuse (NIDA). The facility houses the [Redacted by agreement], which is located and isolated [Redacted by agreement] floor. The facility contains approximately 30,000 gsf of space including animal holding areas and a full array of support rooms.

The APD oversees the NIA animal care and use program and has delegated physical plant oversight to the ARPM. The APD, the ACUC Coordinator, and administrative support offices are located on the [Redacted by agreement] of the animal facility; the ARPM's office is on the [Redacted by agreement] to the animal facility. The Facility Manager, assisted by the Assistant Facility Manager, oversees all animal facility operations; their offices are located within the animal facility.

With a few exceptions on [Redacted by agreement] all NIA animal research laboratories are [Redacted by agreement] on the [Redacted by agreement] floor. The NIDA MRI, used for imaging NHPs, is in the [Redacted by agreement] of the NIDA side of the [Redacted by agreement]

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 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).

1. The animal facility is managed as a conventional facility with a single corridor system and bi-directional traffic pattern to animal housing, procedure, and support rooms. Incoming animals and supplies are received at a Redacted by agreement Redacted by agreement

Access to the facility and general use animal holding and procedure rooms are controlled by Redacted by agreement

Resident animals are transported daily between animal holding and procedure areas within the vivarium. A rodent MRI suite can be accessed through a door exiting off an animal facility corridor. The NIA-side building Redacted by agreement is used to transport rodents and rabbits to laboratories on Redacted by agreement floors of the Redacted A NIDA-side Redacted by agreement is used to transport NHPs to the MRI.

2. The animal facility is located on Redacted by agreement floor, to include some procedure rooms and most behavioral testing areas. There are other animal procedure spaces located in laboratories throughout other floors of the building.

3. Animals are mostly conventionally-housed using shoebox cages or housed within individually-ventilated caging (IVC) systems, using water bottles instead of the rack's built-in sipper systems.

We do have rooms used for isolation purposes for certain incoming rodent shipments, however, these spaces do not meet NIH criteria for "rodent isolation housing" and therefore we do not call them "isolation rooms"; however, in the common understanding of the word they are used at time for isolation purposes (separation of low-to-medium risk shipments until testing

Our BSL-2-ready room possesses a Class II BSC; however, many other of the animal rooms also possess BSCs. Typically only 1 or 2 rooms at most are in use for BSL-2 purposes.

4. Floors:
   - Methylmethacrylate (MMA) in cage wash areas.
   - Troweled, seamless epoxy resin coved to a height of 6 inches at the wall/floor junction in holding rooms, many procedure rooms, and surgery areas.
   - Seamless sheet vinyl in office areas and restrooms

Walls:
   - Level-5 epoxy paint finish over filled masonry block and over gypsum panels and metal frame walls.
   - Wall mounted aluminum guardrails, stainless steel corner guards, and roll bars protect, respectively, centralized facility corridor walls, corners and doorframes.

Ceilings:
   - Level-5 epoxy paint finish over gypsum panels or suspended, scrubbable 2'x4' acoustical ceiling panels and fiberglass reinforced plastic (FRP) tiles in animal housing, testing, and support areas.
   - Suspended, scrubbable 2'x4' acoustical ceiling panels and fiberglass-reinforced polymer (FRP) tiles are used in corridors and cage wash areas, respectively.
   - Water resistant fluorescent lighting banks are recessed.

Doors:
   - FRP doors with hinged observation windows in a heavy gauge stainless steel frame are used for housing rooms and some procedure rooms.
   - Galvanized steel doors and frames are used in all other vivarium rooms; behavioral testing room doors are galvanized sound control doors with galvanized frame.

Behavioral testing procedure rooms are typically outfitted with wall mounted benches or mobile, caster-mounted racks containing open field chambers or other behavioral testing equipment with ancillary computerized data collection equipment; a few behavioral testing areas contain typical, general laboratory closed, wall and base cabinets with countertops for roto-rod, stereotaxic equipment and other tabletop behavioral equipment items. Research staff sanitize primary and secondary enclosures in these areas.

Any areas that are not easily sanitized and how these are maintained:

5. 100% fresh air is provided to the animal facilities after passing through a 30% efficiency pleated pre-filter and 95% efficiency bag-type after filter; air is removed from housing rooms through a 10% efficiency, disposable fiberglass media filter at the individual room level to the exterior at roof level. The HVAC system provides 10 or greater air exchanges per hour in all animal housing and procedure rooms. Air pressure gradients are minimally 50 cfm.

Total supply and exhaust airflow for each animal housing, testing, and procedure room is continuously monitored through an automated building system (ABS).

Animal care staff members monitor air pressure gradient quarterly at rooms' doors using a smoke pen and as needed. Performance data is accessible from the ABS terminal. A work order is generated to evaluate and correct any deviations from the specifications.

The building maintenance personnel perform periodic functional evaluations of the HVAC system no less frequently than every three years. Requested changes in directionality of airflow of rooms are verified by Facilities personnel using the ABS terminal and a smoke pen.

The Redacted by has two types of reheat coil control valves. The electronic reheat coil control valve used within the vivarium fails in the position of last command. The pneumatic reheat coil control valves used in personnel spaces fail in the closed position. The automated building system automatically alerts building engineers of temperature excursions. Building engineers respond by being able to adjustment HVAC operation remotely as well as on-site by rapid recruitment of first-responders.

The ABS system continually monitors supply and exhaust air flows and temperatures of room exhaust air.

Room level control of supply air flow is programmed to trail exhaust air flow to maintain critical negative differential air pressures, e.g. ABSL-2 and quarantine suites, between room and corridor, i.e. negative pressures are preserved as ventilation rates decrease towards a static, neutral pressure state.

The Animal Program's Disaster Response Plan includes coordinated responses by the animal care program staff and building engineers:

- Supply air flow can be reduced at the room level to prevent further excursion in room temperature deviation until correction of a component failure.
- Ventilation can be reduced to conserve conditioned air within a room.

- Rooms can be ventilated through partially opened doors with tempered air from animal facility corridors
- Electric, oil-filled space heaters and spot coolers, which are stored within the animal facility, can be deployed within critical rooms. Additional heaters and coolers are readily available from local commercial sources.
- Hallway ceiling tiles, if needed can be lifted to allow warm air corridor air to escape into the interstitial space between the second and third floor.

6. Physical security support is provided by the Office of Research Services, Security and Emergency Response Services group. Security personnel are stationed at each entrance to the Redacted by agreement Security cameras provide surveillance of parking lots and the building. Cameras are controlled and monitored from a central location within the Redacted by agreement

The entrance to the animal facility is controlled by electronic card readers. Individual rooms have keyed, card reader, or punch code door access systems.

7. There are no exterior windows in animal rooms.

8. Flammable or hazardous agents are stored in safety-approved, storage cabinets in room Redacted by agreement and in procedure rooms within the vivarium. These agents are stored in compliance with NIH safety regulations, as outlined in the BMBL.

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.

We do not have any animal housing areas that meet the NIH definition of "satellite housing areas" as per the Guide, PHS policy, or NIH/ARAC Guidelines.

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.

N/A as per above.

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.

Emergency electrical power is provided for the central animal housing and research areas upon loss of dual feed grid power.

Red colored wall receptacles indicate emergency power protected circuits for animal housing and handling equipment.

Air handlers, ABS control systems, lighting in corridor, housing and procedure rooms, "isolation" and ABSL-2 animal suites, serving ventilated rack blowers are protected by emergency power. Every rack is plugged into an emergency circuit at all times. All other direct animal support systems are also on the emergency power system.

Emergency power is not provided to chillers, therefore environmental temperatures are subject to fluctuations during power outages.

CMS maintains portable cooling and heating units that can be used within animal rooms and be vented directly outside via the house exhaust systems. These may be needed even when racks are on emergency power because the rack blowers generate heat and the overall house air changes will not be able to compensate for that increased heat. Heating units are available in cases when there are HVAC outages during colder weather.

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).

Incidents involving animal welfare are rare. Any such occurrences are reported through OACU to OLAW and AAALAC and included in PHS annual reports and

AAALAC annual reports, as required. There are no such incidents not previously reported.

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.

MRI (rodents): A 7 Tesla magnet in room Redacted by agreement is adjacent to the animal facility and accessible through a door that leads from a hallway within the animal facility. Only rodents are scanned in this room, which is set up and decontaminated according to CMS SOP 6321. Any animals brought from non-NIA facilities must be approved through the rodent import process.

IRRADIATION: Redacted by agreement
Redacted by agreement
Redacted by agreement
based germicidal wipes. All procedures are outlined in CMS SOP 6857 "Irradiating Mice".

MRI (NHPs): We are not currently using the following suite. This suite is only used for NHP work, and we do not currently house or hold NHPs. Anticipated re-start time of this work is 6-9 months:

A 9.4 Tesla Bruckner magnet in the NIDA side of the building is used to scan non-human primates. The suite contains a central MRI room (Room Redacted by agreement with access only through the adjacent control room Redacted by agreement The control room is contiguous with an animal preparation area Redacted by agreement and MRI equipment room Redacted by agreement surrounding Redacted by agreement The MRI suite contains equipment to provide isoflurane anesthesia, to scavenge waste anesthetic gases, to maintain animal warmth, and to ventilate and monitor anesthetized animals, oxygen level alarms, and a quench pipe to passively exhaust cryogen gas, which escaped the MRI magnet core. This room is set up and decontaminated according to NIDA established SOPs. For both MRI suites, SOPs include decontaminating with a chemical agent and covering surfaces with disposable drapes prior to animal entry. Surfaces are decontaminated again once the procedure is completed.

2. Other Animal Program Support Facilities

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

We send blood and other samples to diagnostic pathology laboratories, and utilize rodent quarantine facilities, of the Division of Veterinary Resources, NIH (Bethesda, MD).

We use rederivation services from the National Institute of Diabetes and Digestive and Kidney Diseases, NIH (Bethesda, MD).

We use laboratories of Transnetyx, incorporated, for genotyping services.

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, cancellation, and opposition at: accredit@aaalac.org

Appendix 1: Glossary of Acronyms and Abbreviations

Acronym/Abbreviation	Definition
ACAP	Advisory Committee to the Animal Program
ACUC	Animal Care and Use Committee (NIA version of IACUC)
AF	Animal Facility
AEP	Animal Exposure Program
APD	Animal Program Director
ARAC	Animal Research Advisory Committee
ARPM	Animal Resources Program Manager
ASP	Animal Study Proposal (protocol form)
AV	Attending Veterinarian
AWR	Animal Welfare Regulations
BAS	Building Automation System
Redacted by agreement	
CAPS	Centralized Animal Procurement System
CMS	Comparative Medicine Section
COR	Contracting Officer's Representative
CR	Calorie Restriction
CRL	Charles River Laboratories
DHHS	Department of Health and Human Services
DOHS	Division of Occupational Health and Safety
DRS	Division of Radiation Safety
DVR	Department of Veterinary Resources

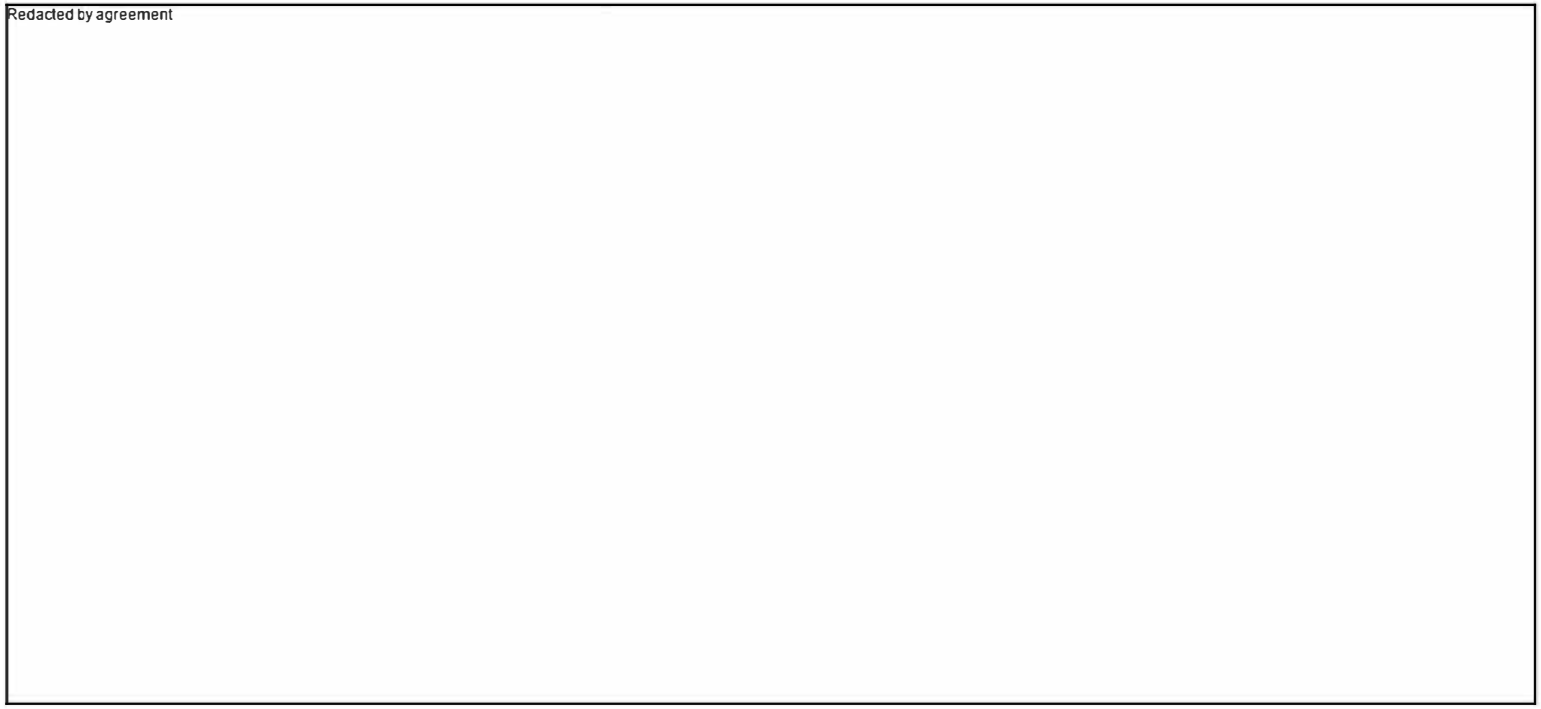
Appendix 1: Glossary of Acronyms and Abbreviations

Acronym/Abbreviation	Definition
FY	Fiscal Year
GSH	George S. Hall, Inc. [Group]
Guide	Guide for the Care and Use of Laboratory Animals (NRC)
HVAC	Heating Ventilation and Air Conditioning
IAA	Interagency Agreement
IASP	Internet Animal Study Proposal [System]
IBC	Institutional Biosafety Committee
IC	Institute or Center
IRP	Intramural Research Program
MOU	Memorandum of Understanding
MPW	Medical Pathological Waste
NHP	Nonhuman Primate
NIA	National Institutes on Aging
NIDA	National Institute on Drug Abuse
NIH	National Institutes of Health
NIHAC	NIH Animal Center [Redacted by agreement]
OACU	Office of Animal Care and Use, NIH
OD	Office of the Director, NIH
OMS	Occupational Medical Service
OLAW	Office of Laboratory Animal Welfare
ORF	Office of Research Facilities

Appendix 1: Glossary of Acronyms and Abbreviations

Acronym/Abbreviation	Definition
ORS	Office of Research Services
OSD	Office of the Scientific Director (NIA)
PHS	Public Health Service
PI	Principal Investigator
PM	Policy Manual (NIH)
PPE	Personal Protective Equipment
SAF	Shared Animal Facility
SD	Scientific Director
SOP	Standard Operating Procedure
T&E	Training and Experience [form]

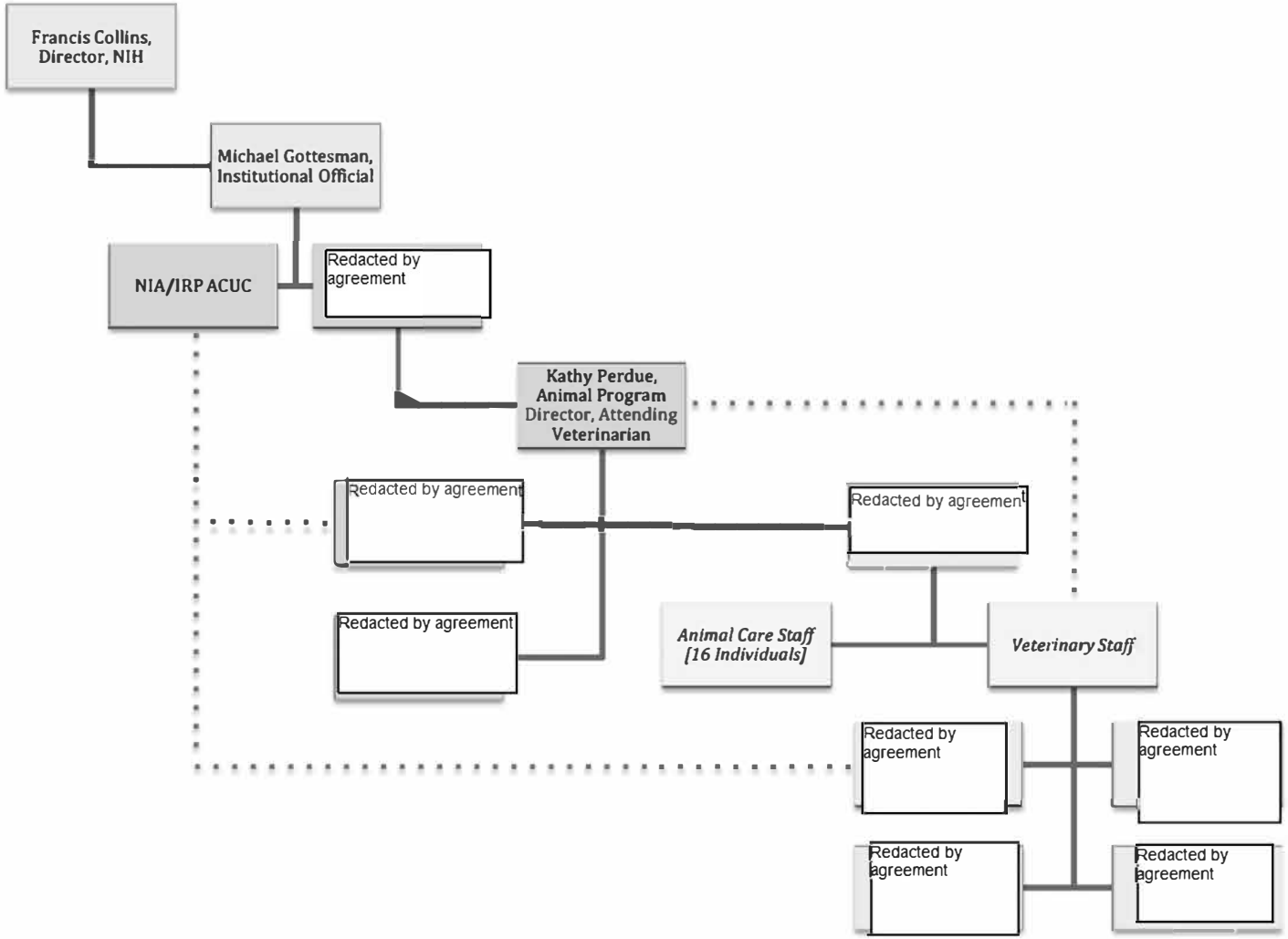
Appendix 2: Summary of Animal Housing and Support Sites



Redacted by agreement

Appendix 3: Line Drawings

Appendix 4: Organizational Chart



Appendix 5: Animal Usage

ASP Number	ASP Title	Principal Investigator	Species*	Total Number of Approved Animals	Pain & Distress Category(ies)	Special Considerations*					
						SS	MSS	FFR	PR	HAU	NCA
Redacted by agreement	The role of dietary and pharmacological interventions on the health characteristics, behavior, and mechanisms in mouse and rat models of neurodegeneration	Redacted by agreement	M, R	3237	C, D					✓	
	Rodent Health Surveillance Program (Sentinel Monitoring)		M, R	2364	C						
	Study of GLP-1 as a prevention or amelioration of diabetes		M	60	C					✓	✓
	Assessment of Primate Aging: Effects of Caloric Modification		NHP	30	D			✓		✓	
	Involvement of the Ectodysplasin (EDA) gene in hair, skin, and tooth fornation		M	19608	C				✓	✓	✓
	Digitalis-like inhibitors of Na pump in Dahl rats in salt-sensitive model of cardiovascular diseases		R	1584	C, D, E	✓	✓		✓	✓	
	Basic Animal Handling and Procedures Training for Investigators and Staff		M, R, GP, NHP, Ra	2192	C, D	✓				✓	
	Neuronal Survival and Apoptotic Mechanisms in Neurodegeneration		R	140	C						
	Investigating the Comparative Physiological and Metabolic Effects of Genetic Interventions in Aging using Dietary Manipulations		M	5028	C, D, E	✓		✓		✓	
	Antibody Diversity in Mice Deficient for DNA Repair Enzymes		M	9029	C, D					✓	✓
	Involvement of the FOXL2 gene in blepharophimosis/ptosis/epicanthus inversus syndrome and premature ovarian failure		M	8280	C						✓
	Forestalling Neurodegenerative Disorders		M	3564	C, D, E	✓		✓		✓	✓
	Purification of cardiotonic steroid marinobufagenin from Bufo marinus toads		T	200	C						✓
	Role of marinobufagenin in salt sensitive hypertension, vascular dementia and aging in rodents		M, R	3312	C, D	✓	✓		✓	✓	✓
	Breeding Protocol for Immune Deficient Mice		M	3960	C, D					✓	
	Immune function analysis using knockout mice		M	2928	C					✓	
	Age and Vaccine Responses to Clinically Relevant Disease		M	13720	C, D, E					✓	✓
	Cancer Escape and Immunotherapy		M	9000	C, D, E	✓				✓	✓
	The Aging Colony: Calorie restriction in mice and rats, Characterization of aging and calorie restriction responses, and Tissue harvest		M, R	3883	C, D	✓		✓	✓	✓	✓

Appendix 5: Animal Usage

ASP Number	ASP Title	Principal Investigator	Species*	Total Number of Approved Animals	Pain & Distress Category(ies)	Special Considerations*					
						SS	MSS	FFR	PR	HAU	NCA
Redacted by agreement	Pre-clinical pharmacology of anti-inflammatory agents and neurotrophic/neuroprotective agents in rodents	Redacted by agreement	M, R	5499	C, D					✓	✓
	Regulation of Immunoglobulin Gene Expression in B Lymphocytes		M	29628	C						✓
	Prevention and reversion of age-associated arterial remodeling by an inhibition of angiotensin II signaling in rats		R	1110	C				✓	✓	
	Breeding and In-Vitro Studies		M	14160	C, E					✓	✓
	Assessment of healthspan and lifespan in mice subject to genetic, dietary and pharmacological interventions		M	1218	C	✓		✓		✓	
	Lifespan of memory T cells		M	3510	C, D					✓	
	DNA repair deficient mice in aging and neuronal degeneration		M, R	13684	C, D	✓				✓	✓
	Holding and Colony Maintenance of NIA Owned Mice, Rats, and Rabbits		M, R, Ra	2340	C, D					✓	
	NF-kappaB, Inflammation and Aging		M	17791	C						
	Nonhuman Primate Holding		NHP	75	C						
	Mouse breeding, maintenance, tissue harvest, and health assessment		M	6000	C, D					✓	
	Age, Genetic, Dietary and Pharmacological Interventions in Mice: The Effects on Cancer Development and Progression in Models of Carcinogenesis		M	280	C, D					✓	
	Protection Against Myocardial Ischemic Injury and Cardiac Remodeling in Rat Coronary Artery Ligation Model		R	720	D	✓				✓	
	Structure and Organization of Memory in the Nonhuman Primate Brain		NHP	11	D					✓	✓
	Early Rearing Condition Effects on Cognitive Behaviors in Monkeys		NHP	27	C					✓	✓
	Role of epigenetic changes in lymphocyte function		M	7254	C, D					✓	
	The impact of mitochondria on longevity in short and long lived inbred mice and their F1 offspring and the response to calorie restriction		M	2976	C, D	✓		✓	✓	✓	
	Neurobiological Markers of Cognitive Aging		R	2012	C, D	✓			✓	✓	
	Effects of Aging on the Proliferative Capacity of Arterial Mural Cells in Rats		R	388	C					✓	
	Evaluating the mechanisms of skeletal muscle regenerative capacity in mice utilizing an experimental immobilization protocol		M	1110	C			✓	✓	✓	

Appendix 5: Animal Usage

ASP Number	ASP Title	Principal Investigator	Species*	Total Number of Approved Animals	Pain & Distress Category(ies)	Special Considerations*					
						SS	MSS	FFR	PR	HAU	NCA
Redacted by agreement	Mouse breeding protocol	Redacted by agreement	M	46336	C					✓	
	Aging Interventions in Rhesus Monkeys		NHP	169	C, D			✓		✓	✓
	Using mouse models to investigate of the role of topoisomerase 3b-TDRD3 complex in Fragile X Syndrome and aging		M	2108	C, D, E	✓				✓	✓
	Pre-clinical pharmacological agents in a model of mild traumatic brain injury (mTBI): effects of age and a repeated mTBI		M	18804	C, D	✓	✓			✓	✓
	Heart and Conduction System Aging in Mice		M	2152	C, D, E	✓				✓	
	Assessing Metabolic Function Across Lifespan in Mice		M	20146	C, D	✓	✓			✓	✓
	Longevity effects in mice fed novel compounds added to high fat and standard mouse chow diets		M, R	1495	C					✓	
	The Role of Milk Fat Globule-EGF8 (MFG-E8) in the Pathogenesis of Hypertension in Mice with Aging		M	1055	C, D	✓		✓	✓	✓	
	Role of regulatory protein IF1 in control of ATP-synthase in mouse heart mitochondria; ATP-synthase function in the IF1-KO (ATPIF1) mouse model		M	623	C						
	Biosynthesis of cardiotonic steroids in rodents		M	2700	C, D	✓			✓	✓	✓
	Studies of immune and autoimmune responses in vivo		M	688	C, E					✓	✓
	Study of inflammation and age-associated immune dysfunction		M	3060	C, D, E	✓				✓	✓
	The Role of Insulin in the Taste Bud Cells of Rodent Tongues		M	10715	C, D	✓				✓	✓
	LCS mouse breeding and calcium homeostasis by rodents, guinea pig and rabbit ASP		M, R, GP, Ra	10896	C					✓	✓
	Study of Longitudinal Aging in Mice (SLAM)		M	2082	C, D	✓		✓		✓	
	Investigation of the role of DNA damage in aging and the impact of dietary and pharmacological interventions to prevent aging and premature aging		M	780	C, D	✓		✓		✓	
	Magnetic Resonance Imaging (MRI) Studies for External Collaboration		M	900	C					✓	✓
	Cell Culture Models for Neurodegenerative Diseases		M	2985	C, D	✓				✓	✓
	Generation and Characterization of Genetically Modified Mouse Models for Neurodegenerative Diseases		M	21000	C, D	✓	✓			✓	✓
	Systems mechanisms of transcription factor dynamics and age-associated epigenomic alterations in immune cells		M	6036	C, D					✓	✓

Appendix 5: Animal Usage

ASP Number	ASP Title	Principal Investigator	Species[▲]	Total Number of Approved Animals	Pain & Distress Category(ies)	Special Considerations[*]					
						SS	MSS	FFR	PR	HAU	NCA
Redacted by agreement	Effect of Aging on Heart Rhythm in Rodents	Redacted by agreement	M, R	2475	C, D	✓				✓	
	Chronic Renal Failure Model in Rats		R	240	C					✓	
	Characterization of hematopoietic stem cell (HSC) and progenitor cell aging, identification of candidate age-regulated genes as targets for therapeutic intervention		M, R	7858	C, D	✓		✓		✓	✓
	Effect of voluntary exercise and aging on heart rhythms and health in rats		R	60	D	✓		✓		✓	
	Thermoregulation and heart rate variability in transgenic mouse models		M	900	D	✓				✓	
	Elucidating the roles of lipid mediators in essential fatty acid deficiency		M	200	C					✓	✓
	Analysis of function of RNA-binding proteins in animal models		M	16224	C, D					✓	✓
	Development and characterization of mouse behavioral tests		M	580	C					✓	
	Analysis of skeletal muscle aging in genetic mouse models		M	5664	C, D					✓	✓
	Parity effect on long-term maternal cardiovascular changes		M	810	C				✓	✓	
	Insulin Signaling in Brain Physiology and Pathology		M, R	10185	C, D	✓				✓	✓
	Mouse Breeding and Colony Maintenance for Cellular Senescence, Aging, and Cancer Studies		M	3276	C, D					✓	
	Studies of RAC1 and FBXW7 mutation status for aging and tumor progression in melanoma and other solid tumors		M	7776	C, D					✓	
	Epigenetic mechanisms of mammalian tissue aging		M	3108	C, D	✓	✓			✓	✓

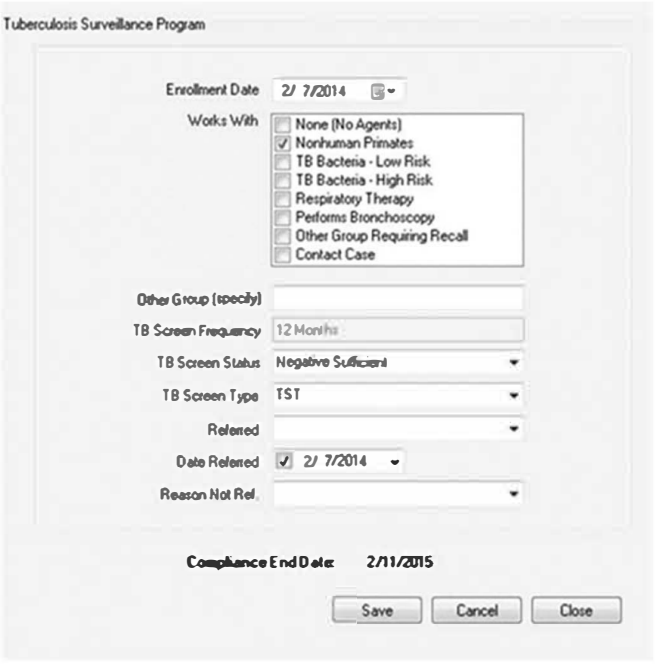
Species	Approximate Annual Use
Mouse	43000
Rat	1100
Rabbit	50
Guinea Pig	40
Nonhuman Primate	150
Toad	None in 2018; 40 in 2017

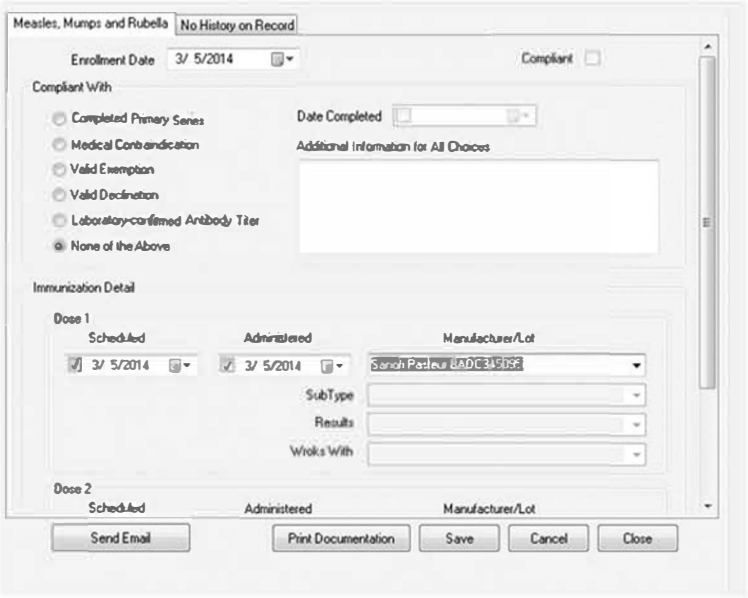
Special Considerations[*]	
SS	Survival Surgery
MSS	Multiple Survival Surgeries
FFR	Food/Fluid Restriction
PR	Prolonged Restraint
HAU	Hazardous Agent Use
NCA	Non-Centralized Procedure Areas

Species[▲]	
M	Mouse
R	Rat
Ra	Rabbit
GP	Guinea Pig
NHP	Nonhuman Primate
T	Toad

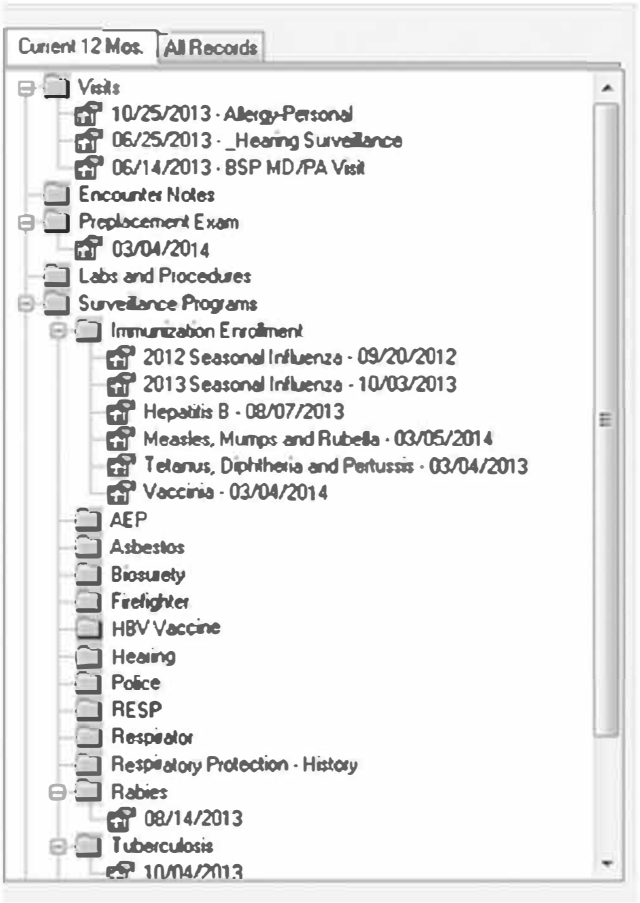
An electronic medical record (EMR) is created at the time of enrollment in the Animal Exposure Program (AEP).

Patient information is also entered into other EMR modules, such as the Tuberculosis Surveillence Program and the immunization log.





Each patient's EMR indexes the associated modules by date.



Appendix 7: IACUC Membership Roster

Name	Degrees/Credentials	Membership Role(s)
Jeffrey Long	Ph.D.	Chair, Scientist
Redacted by agreement		
Kathy Perdue	D.V.M., DACLAM	Attending Veterinarian (Alternate)
Redacted by agreement		

NIA/IRP – ACUC Minutes
Thursday, January 17, 2019
1:02 PM – 2:05 PM, Room 04C211

Members Present: Redacted by agreement Jeff Long, Redacted by agreement Redacted by agreement (via Webex)

Members Absent: Redacted by agreement Kathy Perdue

Observers Present: Redacted by agreement (via Webex)

The Chair, Dr. Jeff Long, called the meeting to order at 1:02 pm.

For those Animal Study Proposals (ASPs) requiring modifications subsequent to full committee review, the standing Designated Reviewers remain the Attending Veterinarian and the Committee Chair. Pursuant to the NIH ARAC Guideline, any member of the ACUC may, at any time, request to see the revised ASP or request full committee review.

The Delegated Reviewers for Appendix and Standard Operating Procedure (SOP) review remain the Attending Veterinarian and the Committee Chair.

New Business:

- Approve December Minutes - The Chair referred the committee to the minutes from the December meeting. The minutes were unanimously approved as written.
- Incident Reports – No new incidents reported.
- ASP Form Discussion – Sections I & J Redacted by agreement

  Section I: Anesthesia, Analgesia, Tranquilization
  This section should include details on any anesthesia, analgesia, or tranquilization used in the ASP. Details should include: Name of drug, dose, route of administration, frequency of administration. Doses should be commonly accepted or published doses. The compounds used must be pharmaceutical grade or justified if not.

  Section J: Method of Euthanasia
  The method(s) of euthanasia must be clearly described in this section. Methods which are not consistent with the recommendations in the *AVMA Guidelines for the Euthanasia of Animals* (2013), must also be scientifically justified.

  CMS SOP6600, "Rodent Euthanasia" can be referenced, which includes the following methods:

  - CO_2 asphyxiation
  - Anesthetic inhalant overdose
  - Injectable anesthetic overdose
  - Cervical dislocation
  - Decapitation
  - Two-stage euthanasia

If this SOP is used, it is necessary to specify which of the method(s) of euthanasia will be used in the ASP.

- Administrative Housekeeping Redacted by agreement

ACUC Member Attendance:

- There must be a quorum (8 voting members) to conduct ACUC business.
- In the event of a scheduled absence that coincides with an ACUC meeting, please let the ACUC Coordinator know by email in advance (i.e. before the agenda for that meeting has been distributed).
- If the agenda has already been distributed, reviewers are still responsible for completing their assigned reviews.
- Last-minute and unscheduled absences are unavoidable, although hopefully infrequent. Please let the ACUC Coordinator know as soon as possible.
- Beginning this year, the NIH awards program will be used to recognize and reward outstanding ACUC members for their attendance, efforts, and participation.
- Committee members are *strongly* encouraged to attend at least one semiannual facility inspection each year.

ACUC Review Timeline:
Two weeks prior to the ACUC Meeting, the agenda and pre-review template are distributed via email. Primary and Secondary reviewer assignments are highlighted on the agenda. Submissions are available through the IASP system.

Reviewers are encouraged to have reviews completed one week prior to the meeting. Reviews should be formatted according to the pre-review template and emailed to the following individuals: PI, Other Reviewer, ACUC Chair, ACUC Coordinator, and Attending Vet.

- ACUC Appendix Review [Long/Perdue]
  - (b)(5) **Disposition: Tabled for Full Committee Review**
  - Appendix 316, "Surgical Procedures" (Triennial Review) **Disposition: Approved**
  - Appendix 327, "Mouse Lens Examination" (Triennial Review) **Disposition: Approved**
  - Appendix 332, "Oral Glucose Tolerance Test and Gavage in Rats and Mice" (Triennial Review) **Disposition: Approved**
  - Appendix 344, "Implantation of Microchips in Rats and Mice" (Triennial Review) **Disposition: Approved**
  - Appendix 358, "Pyruvate Tolerance Testing in Mice" (Triennial Review) **Disposition: Approved**
  - Appendix 360, "Assessment of Blood Pressure in Awake Mice using the IITC Machine" (Triennial Review) **Disposition: Approved**

- Appendix 361, "Assessment of Metabolism in Mice using the CLAM System Under Exercise" (Triennial Review) **Disposition: Approved**

Protocol Business:

1. **Initial Submissions**

   - NONE

2. ***DeNovo* Renewals**

   - [Reviewers: Redacted by agreement (PI: Redacted by agreement LNS) Redacted by agreement "Forestalling Neurodegenerative Disorders" (*cc correspondence to* Redacted by agreement **Disposition: Approved** Redacted by agreement **recused from voting)**
   - [Reviewers: Redacted by agreement (PI: Redacted by agreement LNS) Redacted by agreement "Neuronal Survival and Apoptotic Mechanisms in Neurodegeneration" (*cc correspondence to* Redacted by agreement Redacted by agreement **Disposition: Approved** Redacted by agreement **recused from voting)**
   - (b)(5) (b)(5) **Disposition: Tabled for Designated Member Review** Redacted by agreement **recused from voting)**
   - [Reviewers: Redacted by agreement (PI: Redacted by agreement LCS) Redacted by agreement "Effect of Aging on Heart Rhythm in Rodents" (*cc correspondence to* Redacted by agreement **Disposition: Approved**

3. **Amendments**

   - (b)(5) (b)(5) **Disposition: Tabled for Designated Member Review**

4. **Annual Reviews**

   - (PI: Redacted by agreement LCI) Redacted by agreement "Study of GLP-1 as a prevention or amelioration of diabetes" **Disposition: Approved**
   - (PI: Redacted by agreement LCS) Redacted by agreement "Role of marinobufagenin in salt-sensitive hypertention, vascular dementia and aging in rodents" **Disposition: Approved**

NIA/IRP – ACUC Minutes (continued)

- (PI: Redacted by agreement CMS) Redacted by agreement "Holding and Colony Maintenance of NIA Owned Mice, Rats, and Rabbits" **Disposition: Approved**
- (PI: Redacted by agreement OSD) Redacted by agreement "Aging interventions in Rhesus monkeys" **Disposition: Approved**

5. Notifications

- (PI: Redacted by agreement CMS) Redacted by agreement "Rodent Health Surveillance Program" Amendment #5 – request to update personnel, update emergency treatment form, add the FY2019 testing schedule, and increase rat numbers (<10%). **Disposition: Approved (Administrative)**
- (PI: Redacted by agreement TGB) Redacted by agreement "Pre-clinical pharmacology of anti-inflammatory agents and neurotrophic/neuroprotective agents in rodents" Amendment #4 – request to add blood collection. **Disposition: Approved (Administrative, Veterinary)**
- (PI: Redacted by agreement TGB) Redacted by agreement "Pre-clinical pharmacological agents in a model of mild traumatic brain injury" Amendment #10 – request to add blood collection. **Disposition: Approved (Administrative, Veterinary)**
- (PI: Redacted by agreement CMS) Redacted by agreement "Basic Animal Handling and Procedures Training for Investigators and Staff" Amendment #9 – request to remove personnel and update emergency treatment form. **Disposition: Approved (Administrative)**
- (PI: Redacted by agreement TGB) Redacted by agreement "Pre-clinical pharmacological agents in a model of mild traumatic brain injury" Amendment #9– request to add behavioral task. **Disposition: Approved (Administrative, Veterinary)**
- (PI: Redacted by agreement CMS) Redacted by agreement "Holding and Colony Maintenance of NIA Owned Mice, Rats and Rabbits" Amendment #3 – request to remove personnel. **Disposition: Approved (Administrative)**
- (PI: Redacted by agreement LNG) Redacted by agreement "Generation and Characterization of Genetically Modified Mouse Models for Neurodegenerative Diseases" Amendment #1 – request to add new method of rodent identification. **Disposition: Approved (Administrative, Veterinary)**

The meeting was adjourned at 2:05 pm.

Respectfully submitted,

Redacted by agreement

11 February 2019

Approved.

Redacted by agreement

Jeff Long, Ph.D.
Chair, NIA ACUC
11 February 2019

NIA/IRP – ACUC Minutes
Monday, February 11, 2019
1:01 PM – 2:50 PM, Room 04C211

Members Present: Redacted by agreement Jeff Long, Redacted by agreement Redacted by agreement (via Webex) Redacted by agreement Redacted by agreement (via Webex)

Members Absent: Redacted by agreement

Observers Present: Kathy Perdue, Redacted by agreement (via Webex)

The Chair, Dr. Jeff Long, called the meeting to order at 1:01 pm.

For those Animal Study Proposals (ASPs) requiring modifications subsequent to full committee review, the standing Designated Reviewers remain the Attending Veterinarian and the Committee Chair. Pursuant to the NIH ARAC Guideline, any member of the ACUC may, at any time, request to see the revised ASP or request full committee review.

The Delegated Reviewers for Appendix and Standard Operating Procedure (SOP) review remain the Attending Veterinarian and the Committee Chair.

New Business:

- Approve January Minutes - The Chair referred the committee to the minutes from the Januarymeeting. The minutes were unanimously approved as written.
- Incident Reports – No new incidents reported.
- New Regulatory Veterinarian – The ACUC Program Coordinator introduced Redacted by agreement
- ASP Form Discussion – Sections K & L Redacted by agreement

  Section K: Hazardous Agents

  This section must list any agents/materials that are potentially hazardous to human health:

  - **Radionuclides**: Division of Radiation Safety (DRS) review required
  - **Biological Agents**: Institutional Biosafety Committee (IBC) approval required for bacterial/viral agents, DNA/RNA work, human cells/tissue
    - Must list agent and approved PRD number
    - All biological work will be assigned a BSL by IBC; must note assigned BSL under table
  - **Hazardous Chemicals/Drugs**: any chemical with mutagenic, carcinogenic and/or toxic properties
  - **Recombinant DNA**: IBC approval required
    - Must list approved RD number

The comment section must describe the practices and procedures required for the safe handling and disposal of contaminated animals and materials associated with the study (to include any IBC recommendations).

The use of volatile anesthetics (i.e. isoflurane) requires a description of scavenging methods used.

When applicable, a description of the methods for removal of radioactive waste and the monitoring of radioactivity is also required.

Section L: Biological Material/Animal Products for Use in Animals

Background: Biological material and animal products such as cell lines, tissues, and tumors that are introduced into research animals can harbor animal pathogens (e.g. ectromelia, lymphocytic choriomeningitis and mouse hepatitis) which can then infect NIH animal colonies. PIs are responsible for ensuring that the biologic materials used in their study will not endanger the health of the live animals used in their study or other animals housed in the animal facility.

In this section, the PI describes the materials, the tests performed, and certifies that the material can be used safely in the relevant animal facilities. The documentation of the testing should be submitted as an attachment.

- ACUC Appendix Review [Long Redacted by agreement Perdue]
  - Appendix 301.1, "Dietary and Calorie Restriction in Rats and Mice" (Triennial Review) **Disposition: Tabled for Full Committee Review 01/17/19; Disposition: Approved**
  - Appendix 309.3, "Myocardial Infarction Model" (Triennial Review) **Disposition: Approved**
  - Appendix 309.4, "Vector Delivery to Myocardium" (Triennial Review) **Disposition: Approved**
  - Appendix 309.5, "Rat Model of Diffuse Coronary Disease" (Triennial Review) **Disposition: Approved**
  - Appendix 326, "Non-survival LAD Ligation in Rats" (TO ARCHIVE) **Disposition: Archived**
  - Appendix 346, "Surgical Transverse Aortic Constriction" (Triennial Review) **Disposition: Approved**
  - Appendix 349, "Implantation of Chronic Jugular Vein Catheter in the Rat" (Triennial Review) **Disposition: Approved**
  - Appendix 350, "Surgical Reduction of Renal Mass" (Triennial Review) **Disposition: Approved**
  - Appendix 329, "Active Immunization of Rats against Steroids" (TO ARCHIVE) **Disposition: Archived**

Protocol Business:

1. **Initial Submissions**

   - NONE

2. ***DeNovo* Renewals**

   - (b)(5)

     (b)(5) **Disposition: Tabled for Designated Member Review**

   - (b)(5)

     (b)(5) **Disposition: Tabled for Designated Member Review**

   - (b)(5)

     Disposition: Tabled for Designated Member Review

3. **Amendments**

   - [Reviewers: Redacted by agreement (PI: Redacted by agreement) Redacted by agreement "Basic Animal Handling and Procedures Training for investigators and Staff" Amendment #10 – addition of new species. **Disposition: Approved**

   - (b)(5)

     (b)(5)

NIA/IRP – ACUC Minutes (continued)

(b)(5)

(b)(5) **Disposition: Tabled for Designated Member Review**

- (b)(5)

(b)(5) **Disposition: Tabled for Designated Member Review**

- [Reviewers: Redacted by agreement (PI: Redacted by agreement LCI) Redacted by agreement "The Role of Insulin in the Taste Bud Cells of Rodent Tongues" Amendment #10 – request to modify existing experimental procedure and add experimental procedure. **Disposition: Approved**
- (b)(5)

(b)(5) **Disposition: Tabled for Designated Member Review**

4. Annual Reviews

- (PI: Redacted by agreement TGB) Redacted by agreement "The impact of mitochondria on longevity in short and long lived inbred mice and their F1 offspring and the response to calorie restriction" **Disposition: Approved**
- (PI: Redacted by agreement LBN) Redacted by agreement "Neurobiological Markers of Cognitive Aging" **Disposition: Approved**
- (PI: Redacted by agreement LCS) Redacted by agreement "LCS mouse breeding and calcium homeostasis by rodents, guinea pigs and rabbits ASP" **Disposition: Approved**
- (PI: Redacted by agreement LGG) Redacted by agreement "Analysis of skeletal muscle aging in genetic mouse models" **Disposition: Approved**

5. Notifications

- (PI: Redacted by agreement LCS) Redacted by agreement "Heart and Conduction System Aging in Mice" Amendment #9 – request to add strain and increase animal numbers. **Disposition: Approved (Administrative)**

NIA/IRP – ACUC Minutes (continued)

The meeting was adjourned at 2:50 pm.

Respectfully submitted,

Redacted by agreement

11 March 2019

Approved,

Redacted by agreement

Jeff Long, Ph.D.
Chair, NIA ACUC
11 March 2019

NATIONAL INSTITUTES OF HEALTH
ANIMAL STUDY PROPOSAL
(11/13/2015)

Leave Blank
PROPOSAL # _____
APPROVAL DATE _____
EXPIRATION DATE _____

A. ADMINISTRATIVE DATA:

Institute or Center_____

Principal Investigator_____

Building/Room_____E-Mail_____Telephone_____FAX_____
Emergency Treatment and Animal Care instructions shall be provided on the attached form at the end of this document.

Division, Laboratory, or Branch_____

Project Title_____

_____

Initial Submission [ ] Renewal [ ] or Modification [ ] of Proposal Number_____
List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., Co-investigator(s)): A brief summary of the training and/or experience for procedures each individual will be expected to perform in this ASP must be documented and available to the ACUC. The name(s) of the supervisor, mentor, or trainer who will provide assurance each individual is/has achieved proficiency in those procedures shall be included in that documentation.

B. ANIMAL REQUIREMENTS:

Species_____Age/Weight/Size_____Sex_____

Stock or Strain_____

Source(s)_____Holding Location(s)_____

Animal Procedure Location(s)_____

Estimated Number of Animals:

Year 1	Year 2	Year 3	= TOTAL
_____	_____	_____	_____

C. TRANSPORTATION: Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within the Clinical Center, also include the route and elevator(s) to be utilized.

D. STUDY OBJECTIVES: Provide no more than a 300 word summary of the objectives of this work. Why is this work important/interesting? How might this work benefit humans and/or animals? This should be written so that a non-scientist can easily understand it. Please eliminate or minimize abbreviations, technical terms, and jargon. Where they are necessary, they should be defined.

E. RATIONALE FOR ANIMAL USE: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used. (Use additional sheets if necessary)

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following: (Use additional sheets if necessary.)

Injections, Inoculations or Instillations (substances, e.g., infectious agents, adjuvants, medications, drugs, etc.; dose, sites, volume, route, diluent, and schedules). ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals

Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)

Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)

Radiation (dosage and schedule)

Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)

Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)

Other Procedures (e.g., survival studies, tail biopsies, etc.)

Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain or distress, ascites production, etc.) For Column E studies provide: 1) a description of the procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress cannot be relieved.

Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.

G. SURVIVAL SURGERY - If proposed, complete the following:

None ____ Major_____ Minor_____

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional sheets if necessary):
2. Who will perform surgery and what are their qualifications and/or experience?
3. Where will surgery be performed, Building and Room?_____
4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:
5. Has survival surgery been performed on any animal prior to being placed on this study? Y/N_____ If yes, please explain:
6. Will more than one survival surgery be performed on an animal while on this study? Y/N_____ If yes, please justify:

H. RECORDING PAIN OR DISTRESS CATEGORY - *The ACUC is responsible for applying U.S. Government Principle IV.: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.* Check the appropriate category or categories and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. FOR USDA REGULATED SPECIES, PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA. FOR ALL OTHER SPECIES, THE JUSTIFICATION FOR SUCH STUDIES MUST BE PROVIDED IN SECTION F. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT

NUMBER OF ANIMALS USED EACH YEAR			Year 1	Year 2	Year 3
	USDA Column C	Minimal, Transient, or No Pain or Distress			
	USDA Column D	Pain or Distress Relieved By Appropriate Measures			
	USDA Column E	Unrelieved Pain or Distress			

Describe your consideration of alternatives to procedures listed for Column D and E, and your determination that alternatives were not available. [Note: Principal investigators must certify in paragraph N.5. that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.] Delineate the methods and sources used in the search below. **Database references must include the databases (2 or more) searched, the date of the search, period covered, and keywords used.**

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION: For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration. ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

NONE ___ (check if none)

J. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY: Indicate the proposed method, and if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Guidelines on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

NONE ___ (check if none)

K. HAZARDOUS AGENTS: **NONE** ___ (check if none)

Use of hazardous agents requires the approval of an IC safety specialist.

Biological Agents with Pathogenic Potential: **NONE** ___ (check if none)

For guidance, see ORS/DOHS Biological Safety and Compliance. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Agent:	PRD #:	ABSL:

Additional occupational health and/or animal facility handling safety considerations.

Recombinant DNA: **NONE** ___ (check if none)

For guidance, see NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules FAQs. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Recombinant DNA:	RD #:	ABSL:

Additional occupational health and/or animal facility handling safety considerations.

Ionizing Radiation: (Radionuclides & radiation producing equipment) **NONE** ___ (check if none)

For guidance, see ORS/DRS/Policies/Radiation Safety Protocols Animal Studies Proposal Requirements

☐ Yes, I will use radionuclides or radiation producing equipment as part of the experimental procedures on the ASP and all operators will be registered with Division of Radiation Safety. If an irradiator is to be used, then all individual users must comply with Division of Radiation Safety requirements for irradiator training, and all individual assessors will comply with applicable security requirements for escorts and proxy card access approval.

List of Radionuclides:

Radiological safety considerations:

Hazardous Chemicals or Drugs: **NONE** ___ (check if none)

For guidance, see NIH Policy Manual 3034 – Working with Hazardous Chemicals

Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (Title 29, Part 1910.1200(b)(3)(ii), CFR)

List of Agents:

Additional occupational health and/or animal facility handling safety considerations:

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS: **NONE ___** (check if none)
List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.

Material:	Source:	Sterile? Y	Sterile? N
If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)			
Have the tested materials been passed through rodents outside of the animal facility in question?			
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?			
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens.			

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: **NONE ___** (check if none)
List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. I certify that I have attended an approved NIH investigator training course.
   Month/Year of Initial Course Completion:_____ ; Month/Year(s) of Refresher Training: _____
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (or equivalent, as applicable, for contract personnel).
4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal, have completed the course "Using Animals in Intramural Research: Guidelines for Animal Users" will complete refresher training as required, and received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns. I further certify that I am responsible for the professional conduct of all personnel listed in Section A.
5. **FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section H):** I certify that I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) **as noted in section H,** and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the ACUC before initiating any significant changes in this study.

Principal Investigator:
Signature_____Date_____

O. CONCURRENCES: PROPOSAL NUMBER _____ (LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____Signature_____Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)
DOHS Safety Representative
DRS Safety Representative

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature _____Date_____

Attending Veterinarian: Certification of Review
Name_____Signature_____Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____Signature_____Date_____

NIA
Animal Care and Use Committee
REQUEST TO AMEND AN ANIMAL STUDY PROPOSAL

Protocol Number: **Date**:
Protocol Expiration Date:
Amendment Number:
Principal Investigator:
Protocol Title:

Amendment Summary:

Protocol Number Amendment#

NIA
Animal Care and Use Committee
REQUEST TO AMEND AN ANIMAL STUDY PROPOSAL

Protocol Number: **Amendment #:**
Principal Investigator:
Protocol Title:

Section B: Animal Requirements

Species: Mouse
Change/Addition to the Animal Stock/Strain: *Indicate the strain(s), source(s), age, weight and sex of the animals.*

Age/Unit	Weight/Unit	Sex

Stock/Strain	Source

Change/Addition to the Animal Holding/Procedure Location(s):
Holding Location:
Procedure Location:

Section C: Transportation

Change/Additions to Transportation: Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized.

Section D: Study Objectives

Change/Addition of the Study Objectives: Describe in laymen terms how this amendment affects the aim of the study. Please note that Section D must be completed for all amendments.

Section E: Rationale for Animal Use

1. Explain you rationale for animal use:

2. Justify the appropriateness of the species selected:

3. Justify the number of animals to be used:

4. If applicable, justify why this study uses only animals of the same sex in all experimental groups:

Section F: Description of Experimental Design and Animal Procedures

Change/Addition of a Procedure: Provide detailed description of procedural changes. Indicate any expected adverse affects and treatments. If survival surgery complete Section G. If proposed change(s cause pain and /or distress to the animal(s, indicate any additional analgesics or anesthetics under Section I. Also, provide the database search information by which alternatives to these procedures have been sought under Section H.

Section G: Survival Surgery

[ ] None
[ ] Major
[ ] Minor

Change/Addition of the Survival Surgery Procedure: Describe the surgical procedure(s, including the aseptic methods. Indicate who will perform surgery and their qualifications/experience. Indicate where surgery will be performed. Describe the post-operative care and the responsible individual(s. Indicate if major survival surgery has been performed. Indicate if more then one major survival surgery will be performed.

Section I: Anesthesia, Analgesia, Tranquilization

Addition/Deletion of Anesthesia, Analgesics, Tranquilizers and/or Sedatives: If adding, provide the dosage, route of administration, frequency, injection site and the procedure for which the agent(s) will be used. Also, indicate any hazardous agents under Section K. If the administration is for relief of pain and/or distress to the animals complete Section H.

Section J: Method of Euthanasia or Disposition at End of Study

Change/Addition of Method of Euthanasia: Indicate the dosage and route of administration of any agents used.

Section K: Hazardous Agents

Use of hazardous agents requires the approval of an IC safety specialist.

	List agents and registration document number (if applicable)
[N] Radionuclides	
[N] Biological Agents	
[N] Hazardous Chemicals/Drugs	
[N] Recombinant DNA	

1. Change/Addition of Hazardous Agents:
Be sure to consult with Radiation Safety if adding Radionuclides or with Occupational Safety and Health if adding Biological Agents, Hazardous Chemicals/Drugs, or involves Recombinant DNA.

2. Change/Addition of the Biosafety Level and Safety Considerations:

Section L: Biological Material/Animal Products for Use in Animals

Change/Addition of Biological Material/Animal Products For Use In Animals:

1. Specify material:

2. Source: __

   Is material either Sterile or Attenuated? N/A

3. If derived from rodents, has the material been MAP/RAP/HAP/PCR tested? N/A
   If answer is Yes, please attach copy of results.

4. I certify that the MAP/RAP/HAP/PCR tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.

   _____ Initials of Principal Investigator

Section M: Special Concerns or Requirements

Change/Addition of Housing Requirements: Indicate housing that is not standard for the species or housing which does not meet the animals' physiological or behavioral requirements.

Section N: Principal Investigator Certification

Principal Investigator Signature _____ Date _____

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit and sex as a biological variable. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief.)

Lab/Branch Chief Signature _____ Date _____

For Committee Use Only:

Attending Veterinarian Signature _____ Date _____

ACUC, Chairperson Signature _____ Date _____

If Required:

Occupation Safety and Health Representative Signature _____ Date _____

Radiation Safety Signature _____ Date _____

Facility Manager Signature _____ Date _____

Facility Manager Signature _____ Date _____

Facility Veterinarian Signature _____ Date _____

Facility Veterinarian Signature _____ Date _____

NIA Animal Care and Use Committee
Annual Protocol Review

Protocol Number: _ ***Date:*** _

Principal Investigator: _

Protocol Title: _

[ ] Yes [ ] No 1. *Is this study still active? If* **"No"**, *please send correspondence closing the ASP.*

[ ] Yes [ ] No 2. *Do you use any registered agents (IIPRD, rDNA) under this protocol? If* **"Yes"**, *please submit the required documents to Division of Safety for annual renewal. Copies of the updated documents must be submitted with the annual review.*

[ ] Yes [ ] No 3. *Are there any new developments or unforeseen complications that changed the level of animal pain and/or distress since the study was first approved or the last annual review? If* **"Yes"**, *please describe.*

[ ] Yes [ ] No 4. *Does this protocol involve survival surgery, other procedures, or disease conditions that require post-operative/post-procedural veterinary care? If* **"Yes"**, *please respond to the next question (Question 5).*

[ ] Yes [ ] No 5. *Did animals experience any difficulties during the recovery period that resulted in veterinary nursing care exceeding normal care [e.g. = 20% weight loss, dehiscence of incision (full/partial), infection, paresis/paralysis, dystocia, poor mothering of pups, failure-to-thrive pups, etc.]? If* **"Yes"**, *please describe the steps taken to correct the procedural problems, and report on the number of animals that did and did not respond to the corrective measures taken.*

[ ] Yes [ ] No 6. *Are there any new persons who will conduct research under this protocol? If* **"Yes"**, *please submit an amendment adding the investigators to the protocol before they could perform any procedures on animals.*

[ ] Yes [ ] No 7. *Will there be a change (near future) in Principal Investigator? If* **"Yes"**, *the protocol should be amended to reflect the PI change. A protocol will become inactive if there is no Principal Investigator to oversee the adherence of research on animals to that described in the approved protocol.*

8. *Please provide a progress report on the status of your study. This report should include: a) synopsis of your study to date in narrative form; (b) changes or new directions that have modified the study's approach; (c) explanation of higher or lower morbidity and/or mortality than had been anticipated; and (d) a list of representative publications or presentations during the last year as a result of this research.*

Prinicpal Investigator

Attending Veterinarian

ACUC Chairperson

SEMIANNUAL REPORT

ANIMAL CARE AND USE
PROGRAM REVIEW AND FACILITY INSPECTION
OF THE

National Institute on Aging
Intramural Research Program

OCTOBER 2018

Section A – Site Visits & Program Review:

1. Inspections of the National Institute on Aging (NIA) animal facilities (AF) and areas where any surgical manipulations (Surg) are performed (as applicable) were conducted as indicated below:

Location	Type	Date	ACUC Members
Redacted by agreement	AF, Surg	09/20/2018	Redacted by agreement Long, Redacted by agreement

2. Visits by at least one member of the Animal Care and Use Committee (ACUC) to all remaining areas where animal activities were performed were conducted. These visits occurred during the previous six months and findings and corrective actions are described in this or the previous semiannual report.

3. The following documents were used as the basis for review of the animal care and use program:

	Document/Resource
✓	*Guide for the Care and Use of Laboratory Animals, 8th Edition (Guide)*
✓	NIH OACU "Animal Program Semiannual Assessment Checklist"
✓	Semiannual Inspection Summary

4. The program review was conducted in the following manner:

	Program Review Process
✓	Full committee member review for ALL of the review, i.e. the documents/resources listed in A3. are included in the meeting packet and reviewed at a convened meeting.
	Full committee and subcommittee review, i.e. the documents/resources listed in A3. are assigned to various members who review their parts/sections and then the reviews are discussed with the full committee for a final review/approval.
	Designated member review, i.e. the documents/resources listed in A3. are assigned to various members who review their parts/sections and then report the results of their designated review to the full committee.

Section B – Regulatory Compliance:

Except as noted in Sections F and G, below, the facilities and program are in full compliance with the Public Health Service Policy, the USDA *Animal Welfare Act Regulations (AWARs)*, and the *Guide*, which were used as the basis for this evaluation.

Section C – Program Changes:

The following administrative and procedural changes have occurred since the program was last evaluated:

1. Administrative/Procedural Changes: *None*

2. Key Personnel Changes: *None*

3. Animal Facility/Area Changes: *None*

Section D – Guide Departures & USDA Exceptions:

Departures from the standards of the *Guide,* and exceptions to the USDA *AWARs*, which have been approved by the ACUC, include the following:

1. Departures from the *Guide:*

Guide Departure	Justification
Nonhuman primates on a long-term food regulation study are weighed at least quarterly, instead of weekly. The diet is formulated to ensure that animals receive nutrient levels that meet or exceed the National Research Council's guidelines for NHP nutrition. Food intake and output is monitored twice daily.	Scientific
Mice will be exposed to temperatures outside the *Guide* recommendations (up to 30°C). Although temperatures are outside the regulatory range, they are still within the documented thermoneutral zone for the mouse and are not expected to cause distress.	Scientific

2. Exceptions to the *AWAR: None*

Section E – Previous Deficiencies & Plans:

The ACUC validated that the plans and schedules for deficiencies noted during the previous NIA program review, and facilities and laboratory inspections were achieved within the time intervals projected on the previous semiannual report.

Section F – Current Deficiencies & Plans:

Deficiencies found *over the past 6 months* during NIA program review, facility inspections, and laboratory inspections, are as follows:

#	Deficiency	[1]M/S	Location	Correction Plan	Responsible Party	Scheduled Completion Date	[2]Status: C/P
1	Expired supplies	M	Redacted by agreement	Dispose of expired supplies	Facility Manager, PI	09/20/2018	C
2	Electrical power strip located on floor	M		Elevate power strip	Facility Manager, PI	09/20/2018	C
3	Supplies stored on top of shelving	M		Relocate supplies to provide at least 18" of clearance	PI	10/20/2018	C
4	Electric space heater present in room	M		Remove from room	Facility Manager	09/20/2018	C
5	Secondary containers not labeled with fill and expire dates	M		Label secondary containers with fill and expire dates	PI	10/20/2018	C
6	Overhead canopy is potential fire hazard	M		Remove if not in use	PI	10/20/2018	C
7	Multiple feed trays covered in tape	M		Remove tape and refrain from future use	PI	10/20/2018	C
8	Behavioral equipment dirty from use	M		Clean after each use	PI	09/25/2018	C
9	Miscellaneous items stored in cardboard boxes	M		Move items to sanitizable storage container	PI	10/20/2018	C

[1]M=Minor; S=Significant [2]C=Corrected; P=Pending

Section G – Reportable Events:

PHS Policy (i.e. OLAW) reportable events that occurred in the last 6 months or that are still awaiting final disposition are as follows:

SA First Noted	Description of Event	Status
Fall 2018	A metabolic caging system malfunctioned resulting in elevated temperatures and decreased oxygen levels in the system contributing to the deaths of 12 mice.	Closed

Section H – Shared & Central Facilities:

This semiannual report also encompasses review and oversight of animals and animal activities that were present or occurred in shared or central facilities. Deficiencies were noted and transmitted directly to the facility, and if necessary, to the responsible ACUC. These reviews were conducted as indicated below:

Building	Date	ACUC Members
Redacted by agreement	09/24/2018	Redacted by agreement
	09/25/2018	

Section I – Minority Report:

There ***is not*** a minority report filed with this semiannual report.

NIA ACUC Member Signatures:

Redacted by agreement

Jeff Long
ACUC Chair

Redacted by agreement

Redacted by agreement

Kathy Perdue
Alternate Attending Veterinarian

Redacted by agreement

Appendix 11: 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:	Redacted by agreement

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.

System Details: 100% fresh air is provided to the animal facility after passing through a 30% efficiency pleated pre-filter and 95% efficiency bag-type filter; air is removed from housing rooms through a 10% efficiency, disposable fiberglass media filter at the individual room level and exhausted to the exterior at the roof level. The HVAC system provides 10 or greater air exchanges per hour in all animal housing and procedure rooms. Air pressure gradients are minimally 50 cfm. The HVAC system uses a centralized chilled water system in conjunction with individual room hot water coils and trim humidifiers to maintain room temperature and humidity levels. Total supply and exhaust airflow for each animal housing and procedure room is continuously monitored through an automated building system (ABS). The Redacted by has two types of reheat coil control valves. The electronic reheat coil control valves used within the vivarium fail in the position of last command. The pneumatic reheat coil control valves used in personnel spaces fail in the closed position

System Monitoring: Photoperiod, room temperature, and humidity are regulated through the ABS. The automated system is verified by room thermometer-hygrometers and physical checks of light cycle periods. The ABS provides telephonic and electronic notification to building engineers and animal facility managers of temperature and humidity deviations beyond programmed alarm points. The ABS automatically alerts building engineers of temperature excursions. Building engineers are able to adjust HVAC operations remotely as well as on-site by rapid recruitment of first-responders. The ABS continually monitors supply and exhaust air flows and temperatures of room exhaust air. Room level control of supply air flow is programmed to trail exhaust air flow to maintain critical negative differential air pressures, e.g. ABSL-2 and isolation room, between room and corridor, i.e. negative pressures are preserved as ventilation rates decrease towards a static, neutral pressure state.

Animal care staff members monitor air pressure gradients at least quarterly at room doors using a smoke pen system. Performance data is accessible from the ABS terminal. A work order is generated to evaluate and correct any deviations from the specifications.

The building maintenance personnel perform periodic functional evaluations of the HVAC system no less frequently than every three years. Requested changes in directionality of airflow of rooms are verified by facilities personnel using the ABS terminal and a smoke pen system.

The ABS is monitored at the Office of Research Facilities (ORF)/George S. Hall, Inc. Group (GSH), Central Trouble Call Desk Redacted by agreement at all hours. During normal working hours, 7:00 am to 3:30 pm, Monday through Friday, the animal facility staff is responsible for monitoring the facility; after-hours, 3:30 pm to 7:00 am, and all day on weekends/holidays, monitoring is performed by the Central Trouble Call Desk.

During normal hours, the animal facility staff members monitor environmental conditions and place a service ticket with ORF/GSH for any problems noted.

Emergency Planning: The NIA animal program management and members of the ORF/GSH group have identified and implemented standardized alarm parameters in a two-tier system that specify notification procedures in the event of critical HVAC parameter alarms that could potentially impact animal health (i.e. high temperatures, low air flow). For emergencies, GSH will respond on-site within thirty minutes. During after-hours for a Tier 1 alarm, maintenance staff will respond on-site within 60 minutes of the alarm; no notice is sent to animal facility management. For a Tier 2a alarm, maintenance staff will respond within 60 minutes, if not already on-site; the automated system will contact animal facility management if the room has been at the listed temperature for 2 hours and corrections have not occurred. For Tier 2 alarms, maintenance staff will respond within 60 minutes, if not already on-site; the automated system will contact the animal facility management immediately.

Emergency electrical power is provided for the central animal housing and research areas upon loss of dual feed grid power.

Red colored wall receptacles indicate emergency power protected circuits for animal housing and handling equipment.

Air handlers, ABS control systems, lighting in corridor, housing and procedure rooms, isolation and ABSL-2 animal suites, serving ventilated rack blowers are protected by emergency power. Emergency power is not provided to chillers.

The animal facility's emergency plan includes the following response options:

- Supply air flow at the room level can be reduced to prevent further excursion in room temperature deviation until correction of a component failure.
- Ventilation can be reduced to conserve conditioned air within a room.
- All major cagewash equipment (autoclaves, rack, and tunnel washers) can be shut down.
- Rooms can be ventilated through partially opened doors with tempered air from animal facility corridors; corridor ceiling tiles can be lifted to allow warm air to escape up into the interstitial space once Tier 2 limit is reached.
- Space heaters and spot coolers, which are stored within the animal facility, can be deployed within critical rooms. Additional heaters and coolers are readily available from local commercial sources.

In the Table below, provide room-specific information requested. For each room within this location, indicate use, including the species for animal housing rooms. *Measurement of air exchange rates and verification of relative pressure within animal housing rooms (excluding rooms housing aquatic species only) and cage washing facilities 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*. Information may be provided in another format, providing all requested data is included.

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)**	
Redacted by agreement	Feed/Bedding Storage	65°F	Y	N/A	Y	+	14	03/27/19
	Isolation	72°F	Y	N/A	Y	-	32	03/27/19
	Diagnostic Laboratory	70°F	Y	N/A	Y	-	22	03/27/19
	Necropsy	70°F	Y	N/A	Y	-	51	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	18	03/27/19
	Rat Holding	72°F	Y	N/A	Y	-	16	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	20	03/27/19
	Anteroom & Euthanasia	70°F	Y	N/A	Y	-	74	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	30	03/27/19
	Cagewash (Dirty)	68°F	Y	N/A	Y	-	55	03/27/19
	Cagewash (Clean)	68°F	Y	N/A	Y	-	54	03/27/19
	Sterile Caging Storage	66°F	Y	N/A	Y	+	40	03/27/19

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)	
Redacted by agreement	Procedure Room	72°F	Y	N/A	Y	-	22	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	24	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	20	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	20	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	17	03/27/19
	Rabbit Housing	65°F	Y	+/-3°	Y	-	33	03/27/19
	Procedure Room	69°F	Y	N/A	Y	-	23	03/27/19
	Procedure Room	69°F	Y	N/A	Y	-	21	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	30	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	19	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	23	03/27/19
	Anteroom	72°F	Y	N/A	Y	-	12	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	11	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	11	03/27/19
	Rat Housing	72°F	Y	+/-3°	Y	-	17	03/27/19
	Rat Housing	72°F	Y	+/-3°	Y	-	17	03/27/19
	Rat Housing	72°F	Y	+/-3°	Y	-	14	03/27/19

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)	
Redacted by agreement	Mouse Housing	72°F	Y	+/-3°	Y	-	15	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	14	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	21	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	16	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	24	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	24	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	14	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	20	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	10	03/27/19
	Procedure Room	72°F	Y	N/A	Y	-	21	03/27/19
	Holding (ABSL)	72°F	Y	+/-3°	Y	-	17	03/27/19
	Procedure Room	70°F	Y	N/A	Y	+	17	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	11	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	11	03/27/19
	Procedure Room	72°F	Y	+/-3°	Y	-	23	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	11	03/27/19
	Storage or Housing Swing Room	72°F	Y	+/-3°	Y	NEU	19	03/27/19

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)	
Redacted by agreement	Procedure Room	70°F	Y	N/A	Y	+	15	03/27/19
	Barrier Mouse Housing	72°F	Y	+/-3°	Y	+	14	03/27/19
	Procedure Room	70°F	Y	N/A	Y	+	13	03/27/19
	Rat Housing	72°F	Y	+/-3°	Y	-	17	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	14	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	20	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	17	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	17	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	15	03/27/19
	Procedure Room	70°F	Y	N/A	Y	-	17	03/27/19
	Mouse Housing	72°F	Y	+/-3°	Y	-	16	03/27/19
	Procedure Room	72°F	Y	N/A	Y	+	17	03/27/19

Aquatics Systems Summary:

Not applicable as no aquatic species are used by the NIA/IRP program.

Appendix 13: Primary Enclosures and Space Provisions

Species	Dimensions of Enclosure	Maximum Number Animals / Enclosure	Guiding Document Used (Guide, Ag Guide, ETS 123, Other)	Enclosure Composition & Description**
Mouse	8.75 x 12.125 x 6.395 in	5	Guide	Thoren Maxi-Miser #5 Expanded Mouse Cage, IVCS
Mouse	7.70 x 12.17 x 6.395 in	4	Guide	Thoren Maxi-Miser #9 Small Mouse II, IVCS
Mouse	8.75 x 12.125 x 6.395 in	2	Guide	Thoren Maxi-Miser #15 Single Mouse Duplex, IVCS
Mouse	14.53 x 6.49 x 5.19 in	4	Guide	Tecniplast 1145 Mouse, H-Temp Polysulfone, IVCS
Mouse	> 75 in^2	5	Guide	Lab Products Super Mouse 750, Zyfone, IVCS
Rat	> 210 in^2	5 (400g)	Guide	Lab Products One Cage 2100 – Zyfone, IVCS
Rat	>80 in^2	1 (> 500g)	Guide	Lab Products One Cage – Zyfone, IVCS

Species	Dimensions of Enclosure	Maximum Number Animals / Enclosure	Guiding Document Used (Guide, Ag Guide, ETS 123, Other)	Enclosure Composition & Description**
Rat	>124 in^2	1 (> 500g)	Guide	Tecniplast 1291 Rat Cage, H-Temp Polysulfone, IVCS
Rabbit	61.02 x 33.07 x 72.44 in	1	Guide, AWRs	Tecniplast Stainless steel and Noryl, Multifloor Pen System
Guinea Pig	> 210 in^2	2	Guide, AWRs	Lab Products One Cage 2100 – Zyfone, IVCS

Appendix 14: Cleaning and Disinfection of the Micro and Macro Environments

Area	Washing/Sanitizing Method	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Micro-environment				
Solid-bottom cages (static)	Mechanical Washer	Every week	Citric acid detergent; Alkaline detergent; PH control	Spot changed when needed in addition to scheduled cleaning every week.
Solid-bottom cages (IVC)	Mechanical Washer	Every week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved, spot changed when needed in addition to scheduled cleaning every week.
Suspended wire-bottom or slotted floor cages	Mechanical Washer	Every week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved
Cage lids	Mechanical Washer	Every other week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved
Filter tops	Mechanical Washer	Every other week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved
Cage racks and shelves	Mechanical Washer	Every other week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved
Cage pans under suspended cages	Mechanical Washer	Every week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved

Area	Washing/Sanitizing Method	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Play pens, floor pens, stalls, etc.	N/A	N/A	N/A	N/A
Corrals for primates or outdoor paddocks for livestock	N/A	N/A	N/A	N/A
Aquatic, amphibian, and reptile tanks and enclosures	N/A	N/A	N/A	N/A
Feeders	Mechanical Washer	Every other week	Citric acid detergent; Alkaline detergent; PH control	Autoclaved
Watering devices	Mechanical Washer	Every other week	Citric acid detergent; Alkaline detergent; PH control	RO water is dispensed in bottles.
Exercise devices and manipulanda used in environmental enrichment programs, etc.	Mechanical Washer; Hand Washing (rat running wheels)	Every other week	Citric acid detergent; Alkaline detergent; PH control	Paper tubes, nestlets, and shredded aspen is autoclaved; rubber enrichment devices are not autoclaved
Transport cages	Hand Washing	Between Uses	Chlorine dioxide-based disinfectant	N/A
Operant conditioning & recording chambers, mechanical restraint devices (chairs, slings, etc.)	Hand Washing	Between Uses	Chlorine dioxide-based disinfectant	N/A
Euthanasia chambers	Mechanical Washer; Hand Washing	Between Uses	Citric acid detergent; Alkaline detergent; PH control; Chlorine dioxide-based disinfectant	N/A

Area	Washing/Sanitizing Method	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Macro-Environment				
Animal Housing Rooms:				
Floors	Hand Washing	Daily	Quaternary ammonium-based disinfectant	N/A
Walls	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ceilings	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ducts/Pipes	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Fixtures	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Corridors:				
Floors	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Walls	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ceilings	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ducts/Pipes	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Fixtures	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Support Areas (e.g., surgery, procedure rooms, etc.); complete for each area:				
Floors	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A

Area	Washing/Sanitizing Method	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Walls	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ceilings	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Ducts/Pipes	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Fixtures	Hand Washing	Monthly	Quaternary ammonium-based disinfectant	N/A
Implements (note whether or not shared):				
Mops	Mechanical Washer	Daily	Citric acid detergent; Alkaline detergent; PH control	Not shared
Mop buckets	Mechanical Washer	Daily	Citric acid detergent; Alkaline detergent; PH control	Not shared
Aquaria nets	N/A	N/A	N/A	N/A
Other	N/A	N/A	N/A	N/A
Other:				
Vehicle(s)	Hand Washing	Between Uses	Chlorine dioxide-based disinfectant	N/A
Other transport equipment (list)	N/A	N/A	N/A	N/A

*Please provide chemical, not trade name.

Appendix 15: Facilities and Equipment for Sanitizing Materials

Building	Room No.	Equipment Type	Safety Feature(s)	Methods of Monitoring Effectiveness
Redacted by agreement		Rack washer: BetterBuilt Northstar, Model R690, double-doored walk-in type rack washer between dirty and clean side.	Emergency "off" button; labeled exit door, de-energizing cord on both sides, instructional signage.	Temperature-sensitive tape used twice daily (AM-PM).
Redacted by agreement		Tunnel washer (X2): BetterBuilt Northstar, Model T236, tunnel type washer between dirty and clean side cage wash areas.	Emergency "off" button; and emergency power switch.	Temperature-sensitive tape used twice daily (AM-PM). Monthly HYCHECK swabs on caging components.
Redacted by agreement		Bulk autoclave (X3): Three (3) Getinge Model 182222 AR-2 double doored walk-in type autoclaves.	Emergency "off" button; lock-out key.	Bowie-Dick test done weekly, HYCHECKS on caging components done monthly.
Redacted by agreement		Bottle washer (X2): There are two Edstrom bottle filling stations (Model BF-674). located in clean side cage wash. They are connected to a	Emergency water valve shut-off.	Chlorine PPM test done daily.

Building	Room No.	Equipment Type	Safety Feature(s)	Methods of Monitoring Effectiveness
		reverse osmosis (RO) water purification system that feeds into a chlorine proportioner resulting in water chlorinated at 3-4 PPM.		
Redacted by agreement		Bedding dispensing unit (X2): TBJ, Model BD4800-RH.	Emergency "off" button.	Visual.

Appendix 16: Lighting Summary

Location: Redacted by agreement

Room Type[a]	Light Intensity Range	Lighting Fixture Construction Features[b]	Photo-period (hrs)[c]	Photoperiod and Lighting Control	Override Mechanisms (if applicable)
Rat Holding Rooms	25-30	Water resistant, recessed fluorescent lighting fixtures	12:12	Automatic via building LUTRON management system	One or two fluorescent lamps per fixture provide basal lighting; additional lamps can be activated to provide supplemental lighting in standard light cycle rooms at any time for a defined period of time (15 minutes)
Mouse Holding Rooms	25-30	Water resistant, recessed fluorescent lighting fixtures	12:12	Automatic via building LUTRON management system	One or two fluorescent lamps per fixture provide basal lighting; additional lamps can be activated to provide supplemental lighting in standard light cycle rooms at any time for a defined period of time (15 minutes)
Guinea Pig Holding Rooms	25-30	Water resistant, recessed fluorescent lighting fixtures	12:12	Automatic via building LUTRON management system	One or two fluorescent lamps per fixture provide basal lighting; additional lamps can be activated to provide supplemental lighting in standard light cycle rooms at

Room Type[a]	Light Intensity Range	Lighting Fixture Construction Features[b]	Photo-period (hrs)[c]	Photoperiod and Lighting Control	Override Mechanisms (if applicable)
					any time for a defined period of time (15 minutes)
Rabbit Holding Rooms	25-30	Water resistant, recessed fluorescent lighting fixtures	12:12	Automatic via building LUTRON management system	One or two fluorescent lamps per fixture provide basal lighting; additional lamps can be activated to provide supplemental lighting in standard light cycle rooms at any time for a defined period of time (15 minutes)
Reverse Light Cycle Room	25-30	Water resistant, recessed fluorescent lighting fixtures; Red cellophane sleeves over all bulbs	12:12	Automatic via building LUTRON management system (lights are off from 10:30AM to 10:30PM)	One or two fluorescent lamps per fixture provide basal lighting; additional lamps can be activated to provide supplemental lighting in standard light cycle rooms at any time for a defined period of time (15 minutes)

[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.

Satellite Housing Facilities:

Not applicable as there are no satellite housing facilities in the NIA/IRP program.