Program Description
Animal Care and Use Program

Health Effects Laboratory Division
#000692

Centers for Disease Control and Prevention (CDC) / National Institute for Occupational Health and Safety (NIOSH)

1095 Willowdale Road
Morgantown, WV 26505

December 7th, 2019

For
AAALAC International

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Section 1. Introduction

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.

The Health Effects Laboratory Division (HELD) was established in 1996 as a division of the National Institute for Occupational Safety and Health (NIOSH), an institute of the Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (HHS). HELD is one of three NIOSH Divisions located in Morgantown, West Virginia. Other NIOSH Divisions are located in Washington D.C., Cincinnati, Atlanta, Pittsburgh, Spokane, Fort Collins and Anchorage. Animal based research at this site is done only within HELD.
HELD consists of seven branches: Chemical and Biological Monitoring, (CBMB), Toxicology and Molecular Biology (TMBB), Pathology and Physiology Research (PPRB), Exposure Assessment (EAB), Physical Effects Research (PERB), Allergy and Clinical Immunology (ACIB), and Bioanalytics (BB). Between 1996 and 1999, HELD occupied a new building (L) and expanded in staff size and now employs or provides about 200 people as scientists and support staff. The current animal facilities (AF) were occupied in 1998.

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.

NIOSH's purpose is to conduct research into the causes of occupational illness and develop programs to reduce and prevent work-related illness and injury. NIOSH conducts research from various locations in order to protect workers, and makes recommendations for occupational safety and health standards.

Researchers in HELD conduct focused basic, applied and preventive laboratory research, develop intervention programs, and collaborate with other institutions to apply scientific research to workplace health problems. Some of this research is done at worksites, but most is laboratory research into the disease mechanisms, toxic effects of hazardous substances in the workplace, identifying biomarkers of disease, and developing models of occupational disease.
Animal studies are part of many of these HELD research endeavors. The program of animal care and use is intended to ensure the high quality, proper acquisition and maintenance, and humane use of laboratory animals in these studies.

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 Guide (2011), PHS Policy, and the Animal Welfare Act regulations are used as standards. PHS Policy requires that we base the program of animal care and use on the Guide and comply with Animal Welfare Act-based regulations, utilizing the higher standard when they differ. Other regulations and guidelines, e.g. the AVMA Guidelines for the Euthanasia of Animals: 2013 edition, are incorporated into the primary standards by reference.
July 2020 update: now AVMA Guidelines for the Euthanasia of Animals: 2020 edition

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.

The Associate Director for the Office of Laboratory Science & Safety (OLSS) serves as the Institutional Official (IO) for all of the CDC as delegated from the CDC Director to exercise (1) the responsibility of the CDC Director regarding appointments to the CDC Institutional Animal Care and Use Committees; (2) the authority and responsibility for assuring CDC-wide compliance with all applicable laws, regulations, policies, and standards regarding the humane care and use of laboratory animals at CDC; and (3) to serve as the IO for purposes of compliance with the PHS Policy on Humane Care and Use of Laboratory Animals and relevant regulations issued by the U. S. Department of Agriculture under the Animal Welfare Act (9 CFR Parts 1 - 3). The Chair of the IACUC and the Attending Veterinarian (AV) both have direct lines of communication with the IO by phone and email. The current Associate Director for Laboratory Science & Safety at the CDC is Stephen S. Monroe, PhD. He in turn delegates regulatory compliance oversight to his OLSS Director of Policy, Linda Pimentel, DVM, who directs the CDC's Animal Care and Use Program Office (ACUPO).

The Division Director of the Health Effects Laboratory Division, National Institute for Occupational Safety and Health (Morgantown, West Virginia) has responsibility for the animal care program at this institution. He directs the scientific effort for the institution, and all participating scientists report through their Branches to him. He in turn delegates to the Animal Facilities Director/Attending Veterinarian and staff the day-to-day operation of the animal care program. The current Director is Donald H. Beezhold, PhD.

The Animal Facilities Director (AFD)/ Attending Veterinarian (AV) (b)(6) DVM reports to the HELD Director but has an open line of communication with the IO. The IACUC Administrator (IA), a relatively new addition to the program, reports to the HELD Director as well.

The IA, Chair and AV form the IACUC Administrative Group. The Administrative Group teleconferences with the ACUPO throughout the year and communicates with them regularly on policies and compliance.

Contracted animal facility staff are supplied by (b)(4) and the contract is administered by (b)(6) PhD). The AF Supervisor (b)(6) BS, LATG, CMAR works closely with the AFD/AV for day to day operations of the animal facility. There is one Supervisor, 7 animal care technicians (ACT) and 4 inhalation technicians (IT).

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.

Atlanta based personnel (will likely join by Skype)-
Stephen S. Monroe, PhD - Institutional Official, CDC
Linda C. Pimentel, VMD, MPH, DACVPM, Animal Care and Use Program Office Chief

Morgantown based personnel -
Don Beezhold, PhD - HELD Division Director
Paul Siegel, PhD – HELD Associate Director of Science
(b)(6), DVM, DACLAM - Animal Facilities Director & Attending Veterinarian
PhD - IACUC Chair
(b)(6) MS - IACUC Administrator
MS Industrial Hygiene – IACUC member/ Safety Office Representative
(b)(6) BS, LATG, CMAR, (Contractor) - Animal Facility Supervisor
(b)(6) Animal Facility Operations Officer
Linda Benson, - Laboratory Quality Coordinator
Steve Fotta – Building Manager, Office of Facilities Management

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.

HELD's research and testing involving animals includes toxicology in the broadest sense, from molecular to whole-animal studies of adverse effects resulting from hazardous substances, agents, and practices. It also involves development of models for occupational diseases and exposures, study of the integration of organ systems in response to hazardous or deleterious conditions, establishment of biomarkers for detecting susceptibility and disease, and research on cellular mechanisms involved in disease processes. There are currently no teaching programs using animals, although the AV is planning to soon write a protocol using extraneous animals to train personnel in animal handling and techniques. There are over 40 principal investigators involved with 66 animal protocols active in FY2019. This program uses rodents (in-house breeding colonies and purpose bred, commercially available rats, mice and guinea pigs) only.

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

All research funding is allocated from the U.S. government, primarily through the Department of Health and Human Services / Public Health Service (PHS) budget. Small portions of the research are funded through Interagency Agreements with other government agencies such as the National Toxicology Program (NTP)/NIEHS, CPSC, and Department of Defense. Research funding for HELD investigators, support staff, equipment, supplies, etc., totals approximately $31.5 million per fiscal year, of which about $1.3 million is used for animal purchases, care, maintenance, supplies, equipment, and professional staff.

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.

Not applicable

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.

Onsite contract personnel for animal care and inhalation technicians are supplied by (b)(4) (b)(4) This contract expires the end of January 2022. (b)(4) is not AAALAC accredited, however the contractor employees work in our AAALAC accredited facilities and oversight is provided by the Animal Facilities Director/Attending Veterinarian. (b)(4) provides surgically altered rats that are used on one protocol, in which telemetry devices are surgically implanted intra-abdominally. The animals are transported after recovery to NIOSH. The (b)(4) holds an OLAW Assurance, USDA registration and is AAALAC accredited. Copies of the (b)(4) telemetry implantation surgical procedure, Peri-Operative Care SOP, and the contract between (b)(4) and NIOSH are available upon request during the site visit. (b)(4) was contracted in 2019 to breed a small number of CMT1a transgenic rats we shipped them from our facility, collect embryos and cryopreserve them. The production of embryos has been completed, and the contract will remain active for another two years of cryopreservation storage.

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

Nothing to add.

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.

CDC's Associate Director for Laboratory Science & Safety serves as the Institutional Official (IO) for all of CDC, as delegated by the CDC Director. The IO has responsibility for making appointments to the IACUCs, responsibility and authority for assuring CDC-wide compliance with regulations regarding animal care and use, and serves as IO for the purposes of compliance with the PHS Policy and USDA regulations. The Chair of the IACUC and the Attending Veterinarian have direct lines of communication with the IO by phone, email, etc. as needed, and their reports to OLAW or other agencies are made through the IO. The IO and his staff last visited this site in June 2019.
The Division Director of the Health Effects Laboratory Division, National Institute for Occupational Safety and Health (Morgantown, West Virginia) has responsibility for the animal care program at this institution. He in turn delegates to the Animal Facilities Director / Attending Veterinarian and staff the day-to-day operation of the animal care program. The current Director is Donald H. Beezhold, PhD.

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.

The Attending Veterinarian is (b)(6) DVM, DACLAM. He has a full time position dedicated to the animal care program.

Responsibilities are the health and well-being of all laboratory animals, including veterinary care, animal husbandry and facility management. He advises the IACUC and Division leadership on regulations that pertain to the HELD program, including best practices in the industry. He also communicates to the Division Director on a weekly basis the operational status and any support needs in his role as the Animal Facilities Director. He has delegated responsibility for overall management of the animal care program with operational oversight of animal care staff including access to all research animals.

The key elements to the AV's responsibilities are:

- Animal procurement and transportation
- Disease surveillance, prevention, diagnosis, treatment and control
- Handling/restraint, anesthesia, analgesia and methods of euthanasia
- Surgery and perioperative care
- Assessment and promotion of animal welfare and enrichment
- Development of humane endpoints
- Assessing the adequacy of the animal husbandry program
- Veterinary review and approval of all animal care and use as a primary IACUC member
- Training of institutional staff in the care and use of laboratory animals
- Development of biosecurity policies within the animal facility
- Advising the Safety Office on the Occupational Health Program for animal users

The AV, IACUC Chair and Administrator form the IACUC Administrative Group, and they meet regularly to discuss programmatic issues.

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.

(b)(6) - Animal Facility Operations Officer; places animal orders, orders and tracks veterinary supplies, drugs and equipment, and manages AF Working Standards. He reports operationally to the AV and will be detailed to the Office of the Director (OD) administratively as of Jan 2020.
(b)(6) Veterinary pathologist: assists with diagnostic cases and as back up veterinarian. (b)(6) is also a research scientist in PPRB and reports to the branch chief.
(b)(6) - Veterinary pathologist: assists with diagnostic cases and as back up veterinarian. She also reports to the branch chief of PPRB.
Animal Care and Inhalation Staff – Identify health issues, report health issues and conduct treatments as directed by the AV. As contractors, they report to their on-site supervisor who reports to the (b)(6) who administers the contract. The AV is a Project Officer on the contract and works with the AF Supervisor on day to day operation of the AF.

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.

There are currently two inter-institutional collaborations using live animals. Dr. (b)(6) (b)(6) has an MOU with (b)(4) for collaboration in an NTP (National Toxicology Program) funded project to study the effects of fungal and mold spore inhalation exposures in mice. For this collaboration all animal housing and experiments are conducted in the NIOSH HELD facilities under the

NIOSH IACUC approved protocol 19-016. (b)(6) has a service contract with (b)(4) to perform Two-Photon imaging on mouse lungs exposed to multiwalled carbon nanotubes (MWCNT), C60Fullerene, and silica or asbestos. The ACUPO issued a Letter of IACUC Deferral in June 2019 for this work to proceed under (b)(4) IACUC approved protocol #20160279, and all work is conducted in their facilities using their animals. (b)(4) program is PHS Assured and AAALAC accredited.

A renewed Affiliation Agreement between NIOSH and (b)(4) (b)(4) was signed into effect on 5 November 2019. This agreement encourages collaborative efforts that may include research, service and clinical/professional training. (b)(4) faculty may be appointed as NIOSH Guest Researchers (volunteers), subject to all rules and regulations governing NIOSH research activities. NIOSH personnel may become involved with animal research conducted at (b)(4) Per this agreement such animal use by NIOSH personnel, once approved by the (b)(4) IACUC will not require additional review and approval by the NIOSH IACUC. Record keeping and continuing review of such projects will be the sole responsibility of the (b)(4) IACUC. For animal research, training, or testing conducted at NIOSH- or the use of animals owned by NIOSH where the (b)(4) faculty, staff, or students are involved- prior review and approval by the NIOSH IACUC and the (b)(4) IACUC is required. Record keeping and continuing review of such projects will be the sole responsibility of the NIOSH IACUC, with (b)(4) maintaining its own records.

2. Personnel Management

a. Training, Education, and Continuing Educational Opportunities

Describe *how* the IACUC/OB provides *oversight* and *evaluates the effectiveness* of training programs and the assessment of personnel competencies. Describe how training is documented.
Note: Do not include details about the training program, which should be described in the following sections.

The IACUC evaluates the effectiveness of the training program during semi-annual program reviews. Most of the didactic training is provided through the AALAS Learning Library (A.L.L.), and Animal Users must pass the test for each module

assigned indicating mastery of the subject. A determination is made if the coursework in the A.L.L. has sufficient scope, if the content is appropriate, and if the frequency of retraining is sufficient. Open ended questions are developed as part of the laboratory semi-annual inspection, and laboratorians are asked these questions during the visit to determine understanding of concepts related to animal use. PAM is being used with increased frequency to assess training, particularly with procedures associated with greater potential for pain/distress. All training is documented by the individual on a form that is forwarded to the IA who maintains a file on the shared drive.

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.

(b)(6) - Animal Facilities Director & Attending Veterinarian in HELD, and NIOSH agency representative to ICCVAM since October 2018. (b)(6) holds a BS degree in Veterinary Medicine and a DVM from the University of Illinois (1990) and an MPH from the Uniformed Services University of the Health Sciences (1999). Prior to coming to NIOSH, he worked for the Walter Reed Army Institute of Research and a small CRO following a career in the U.S. Army Veterinary Corps. He completed a combined residency in public health and laboratory animal medicine in 2001 and earned board certifications with the American Colleges of Veterinary Preventive Medicine (1999) and Laboratory Animal Medicine (2001). He holds a West Virginia controlled substance permit and a DEA registration. He is licensed to practice veterinary medicine in Illinois and maintains USDA category II Accreditation. He completes at least 25 hours of continuing education annually to maintain veterinary licensure and board certification. Continuing education attendance includes national AALAS meetings, TRBAALAS meetings, AALAS Learning Library, online (e.g. JAX, OLAW) webinars, and USDA-APHIS online accreditation training.

(b)(6) – Research Veterinary Medical Officer and scientist in the PPR Branch of HELD and a NIOSH employee for 27 years. From 1992-1998 and from May 21 -October 8, 2018, she served as the NIOSH AV. (b)(6) is both an independent and collaborative researcher and is available to assist in the work up of clinical cases that arise in the animal facility. She is a licensed to practice veterinary medicine in Texas and West Virginia. (b)(6) completed her veterinary training at Texas A&M University (1981), received a Master's degree from Purdue University (1986) and completed a residency in veterinary pathology (1988) and PhD (1989) from Colorado State University. She became a diplomate of the American College of Veterinary Pathologists in 1991. (b)(6) completes at least 18 hours per year of continuing education in veterinary medicine and pathology to maintain licensure. Meetings attended in the last 3 years include the Society of Toxicologic Pathologists (STP) Annual Meeting (2017, 2018, 2019), the National Toxicology Program Satellite Symposium to the STP meeting (2017, 2018, 2019), the AKC Canine Health Foundation Semi-Annual Symposium (2017), the RTP Rodent Pathology Course (2019), and several online courses from multiple RACE-approved providers (VIN, IDEXX, Pet Poison Helpline, others).

(b)(6) – Research Veterinary Medical Officer and veterinary pathologist in the PPR Branch of HELD since 2014. Prior to joining NIOSH, Dr. (b)(6) was a veterinary pathologist in the Comparative Medicine Branch at NIH. (b)(6) received her veterinary degree from Iowa State University (1995), a PhD from University of Florida (1999) and completed a residency in veterinary pathology at Louisiana State University (2010).

(b)(6) – Is the Clinical Veterinarian and Associate Director in the Office of Laboratory Animal Resources at (b)(6) He completed his veterinary degree at Cornell University (1972) and is an ACLAM diplomate. He has many years' experience in laboratory animal medicine including primates, ABSL-3 rodents and teaching in the UPR Veterinary Technician program, UVA and WVU. He recently completed a term as an ad-hoc specialist for AAALAC. He regularly attends

AALAS national and local branch meetings. (b)(6) is available for back up emergency veterinary care for NIOSH, when necessary.

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

1) Indicate the number of animal care personnel.

Animal Facility Supervisor
7 Animal Care/ Husbandry Technicians
4 Inhalation Technicians
All of these individuals are currently supplied through contract by (b)(4)

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.

A summary of the education background and years' experience is provided in Appendix 18. Training for new contractors is provided by the Animal Facility Supervisor (AFS) and consists of shadowing and mentoring. The new hires are also required to complete basic coursework in the ALL as well as meet all CDC/NIOSH mandated security and safety training. New hires are required to obtain at least ALAT certification at the completion of the first year. On a rotating basis, all animal husbandry personnel are provided an opportunity to attend the national AALAS meeting and dues for the local AALAS branch are paid for as long as they are active members. Training is also accomplished during regular staff meetings, and informal presentations are made by individuals that attend the national meeting. Vendors are also permitted to provide training if applicable on such topics as breeding colony management, behavior, enrichment, sanitation and disease surveillance.

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.

All personnel who perform procedures on live animals are required to be named on an approved animal care and use protocol with the exception of the animal care and inhalation facility staff, whose training is monitored centrally by the IACUC.
Any new personnel who will be participating in animal research are identified by their supervisor by annotating this activity on their Employee Training Checklist, also called the New Employee, Fellows and Facility user (NEFF) form. This form is updated by the supervisor if the person's job duties change. A copy of the form is sent to the AV and IA which prompts the IA to send an onboarding email describing their training requirements and providing an electronic form to fill in training and experience relevant to the work that they will perform with animals at NIOSH. The AV may assign additional training specific to certain procedures like anesthesia. The AV communicates with the Safety Office Technician who enrolls the individual in the Occupational Health Program for Animal Users. Once the training is completed, the individual is oriented to the animal facility by the AF Supervisor. Once this step is complete and the Occupational Health Clearance form is received from the Safety Office, the AV or IA approves access to the AF and the individual is added as a protocol associate to the protocol(s) requested. The PI is responsible for ensuring that all individuals on their protocol are trained and proficient in live animal procedures as necessary. Training and experience are captured on standardized forms which are forwarded to the IA when updated. Post approval monitoring (PAM) is performed by the AV or subject matter expert (SME) for more invasive procedures such as surgery and category E studies. The AF staff are also required to report to the AV/AFD if there are any concerns related to how animals are handled, which is a form of PAM.

a) Briefly describe the content of any required training.

Didactic training is provided primarily through the A.L.L. Courses for all users include *Working with the IACUC* and *Laboratory Animal Allergies* every 3 years. Species specific courses including *Introduction to-* and *Working with Rats, Mice and Guinea Pigs* depending on the species in the protocol; and *Post-procedure Care of Mice and Rats in Research: Minimizing Pain and Distress*. If anesthesia and/or surgery are described in the protocol then additional modules including *Aseptic Techniques for Rodent Survival Surgery, Inhalation Anesthesia Systems for Rodents* and *Pain Management in Laboratory Animals* may be assigned by the AV. The Working with the IACUC module in A.L.L. covers the regulations, alternatives, unnecessary duplication, humane endpoints, surgery, occupational health, personnel training, euthanasia, protocol modifications, reporting animal welfare concerns, non-compliance and the IACUC. During AF orientation with the AFS, entry requirements are covered in addition to the tour to understand the layout. Highlights of the tour include room signage, how to locate PPE, documenting visits in the room log book, how to work with the animal caging, how to ensure welfare when animals are returned to the housing unit and how to report problems or find assistance.

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

Documentation of training is required before new personnel are added to a protocol and allowed AF access by the AV. During protocol or amendment submission, training records are reviewed to ensure they are up to date. The protocol or amendment will not be approved until the update is completed and the documentation received by the IACUC Office.

c) Describe continuing education opportunities offered.

The Working with the IACUC and Laboratory Animal Allergy modules in ALL are required to be retaken every 3 years. Additional modules may be reassigned if the content changes significantly as was the case with the

2013 revision of the AVMA Euthanasia Guidelines. "All Hands Meetings", a division staff meeting scheduled quarterly, is a platform used by the AV, IA or IACUC Chair to provide programmatic updates.

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]

Personnel performing surgical procedures must be identified in the protocol. Their training records are reviewed by the AV and additional training both didactic and hands on is provided based on their experience and the invasiveness of the procedure. Personnel that perform surgery are monitored by the AV. The PI is responsible for ensuring that personnel working on their protocol are identified, trained and proficient. They attest to this when they sign their protocols. Training records are reviewed by the IA and AV.
One Investigator contracts with a vendor to perform surgical implants in rats; the vendor is AAALAC accredited and has an OLAW assurance.

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

New personnel using anesthesia machines are required to complete the A.L.L. module *Inhalation Anesthesia Systems for Rodents* and to read the SOP for the Somno-Suite system, if applicable. The AV then observes the person performing the procedure ensuring they understand the basic functions of the anesthesia machine, how to monitor and recover the animal. Personnel using isoflurane in bell jars (or similar) and injectable agents are trained by other experienced users in their group, or the potential user can contact the AV or IA to identify a subject matter expert to assist with training.

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

All users must complete the A.L.L. Euthanasia module and pass the exam. The module must be repeated when there are significant updates as was the case with the AVMA Guidelines for the Euthanasia of Animals. The PIs assure that all personnel on their protocol are trained. The SOPs and policies on euthanasia also emphasize that personnel must be proficient in animal handling, injection and technique with respect to physical methods such as conscious decapitation. The majority of experimental animals are euthanized by overdose of barbiturate followed by a secondary method, whereas most breeding colony and sentinel animals are euthanized by CO2 asphyxiation. Physical means, bilateral thoracotomy and cervical dislocation, are utilized as secondary methods to ensure death. New personnel receive hands on training to ensure proficiency in these methods, and retraining is provided when the need is identified. New personnel being trained for physical methods must make use of cadavers to hone technique before moving on to live animals.

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 Safety and Health Program (OSHP) for all personnel with significant animal exposure is administered by the Safety Office, which resides within the Office of Facilities Management Office (OFM). The Safety Office

takes the lead to provide training, oversight and consultation with respect to occupational health and safety, chemical safety, biosafety, radiation safety and biohazardous waste disposal for the animal care program. OFM has oversight of the Health and Safety Office and Facilities Operations, which includes contracted housekeeping and security personnel. The Safety Office contracts with Worksite Medical, Ellwood City, Pennsylvania, to provide health professionals to review health history questionnaires submitted by personnel with significant animal contact and for vaccinations. For animal husbandry and inhalation contract personnel, the health history evaluation and physicals are completed through the contracting company/vendor.
The Safety and Radiation Safety Committees meet at least quarterly.
The Safety Office staff includes an Industrial Hygienist who serves as a full voting member of the IACUC and reviews all new protocols and protocol modifications. His alternate is the Safety Manager in the Safety Office and has a background in Safety Management. A Safety & Occupational Health Specialist with an Immunology and Microbiology background is heavily involved in the required Safety Training classroom courses, which are offered multiple times throughout the year, and she supervises the chemical safety inventory and disposal program. A Technician II position is contracted to support the OSHP, and she holds a Master's Degree in Public Health.

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.].

New processes are evaluated by the Safety Office to assure compliance with regulatory guidelines. Additionally, through an onsite chemical inventory database, the Safety Office is apprised of new chemicals coming into the facility on a weekly basis. Job hazard analyses are developed for high hazard chemicals and made available through the facility intranet. All new employees

must complete a new employee safety orientation prior to receiving their facility access badge. Badged contractors must also complete new employee safety training. Visitors to the site must be escorted by an onsite badged employee. Quarterly facility wide safety audits are completed with findings tracked to completion and trended to identify any new areas of concern.

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

Job hazard analyses are developed for high hazard work or work involving the use of high hazard chemicals. Quarterly facility wide safety audits are completed with findings tracked to completion and trended to identify any new areas of concern. Any changes in existing processes that may alter the handling, use, or storage of existing hazards are reassessed for safety.

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]

All work related incidents and near misses are investigated utilizing a team approach to include a member of the Safety Office. All incident investigation team members have been trained by the National Safety Council. Incidents and near misses are logged on a SharePoint site and tracked for trends. NIOSH has been a Voluntary Protection Program (VPP) Star site since 2005, which involves submitting a report to OSHA annually, and is inspected for recertification every five years.

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.

New hires for NIOSH are identified by their supervisor as animal users. This prompts an onboarding process which includes the Safety Office, IA and the AV. The Safety Office Technician sends the employee a health questionnaire packet electronically which the employee completes and sends electronically directly to the contracted health professional for review. When the review is complete, the health professional sends a clearance form back to the Safety Office Technician who forwards a copy to the IA. This review is updated every 5 years or more frequently if the employee experiences a change in their health status. The program covers all personnel working with research animals identified on an ACU protocol, including contractors and students from (b)(4). Exceptions can be granted for (b)(4) personnel if they are covered under the (b)(4) program and provide documentation. Personnel who perform necropsies are also covered based on their exposure.

Facility Operations and Maintenance staff and Security staff (contractors) were determined not to have significant animal contact but do receive an orientation to the animal facility when hired and sign off on a document stating they understand the potential exposures in the animal facility. Housekeeping staff which are contracted do not enter the animal facility and may only enter laboratories with permission to perform non-hazardous housekeeping tasks and are therefore considered low risk.

Animal and Inhalation facility contracted staff undergo annual physicals and health history evaluation by a 3rd party vendor contracted by their employer. The health professional that performs these evaluations completes the same clearance form described above, which is returned to the contractor Supervisor and forwarded to the Technician in the Safety Office. As above the Safety Office forwards this clearance form to the IA.

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

All animal users are required to complete a health questionnaire which is reviewed by a medical professional. All animal users listed on protocols, animal caretakers, inhalation technicians and histopathology prosectors are enrolled in the NIOSH or employer (contracted staff) occupational health program for animal users.

c) Describe provisions for assuring confidentiality of medical information.

Personnel are emailed the blank form and directed to return the completed form electronically to the health professional (contractor) performing the evaluation. The only document NIOSH receives from the contractor is whether or not they have been cleared to work with animals or need an accommodation such as wearing a respirator.

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

The laboratories and animal facility are secure access and require a badge for entry.
Visitors to the animal facility, including service personnel, are required to review and sign a document stating potential risks. Visitors to the animal facility are not allowed direct access to animal or procedures rooms. Service personnel (either contracted or facility operations) are provided PPE appropriate to the task and exposure potential. For entry into the animal room, scrubs or coverall are required and also include hair bonnet, surgical mask, gloves and shoe covers.
All employees with access to laboratories must complete appropriate safety training. All lab corridors where animals may be temporarily located are

posted to inform personnel that laboratory animals may be present in the laboratory corridor.

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.

Health evaluations are performed as part of the onboarding process for new animal users which occurs prior to the individual being named on any protocols, performing procedures on animals and obtaining access to the animal facility. NIOSH personnel with significant animal contact complete a health history questionnaire which is reviewed by a health professional contracted by the Safety Office. The medical evaluation is performed every 5 years or more often if the individual's health status changes. There is also an extensive Medical Surveillance Program offered to all NIOSH personnel which includes services such as physical examination, blood chemistries, complete blood count, urinalysis, chest radiographs, electrocardiogram and auditory testing at no charge provided by an outside contractor that visits the site. This program includes facility maintenance personnel.
Animal Facility contracted personnel undergo an annual physical exam and health history evaluation to determine 'fitness for work' by an Occupational contractor chosen by their employer; this includes a requirement to be up to date on tetanus vaccination.
No diagnostic testing is required for either program based on species used or exposures.
NIOSH has not and is not currently using species considered potentially hazardous. The animal research program uses commercially available mice

and rats almost exclusively. Other sources of mice and rats would almost always go through a rederivation procedure to ensure their health status is compatible with that of our resident populations. All of these animals would have a history of testing negative for zoonotic agents and be maintained in such a manner to prevent introduction of these agents thereby minimizing the risk to personnel. There is no immunization requirement; however, in the event of an animal bite or an injury where an individual's tetanus status is questionable, we will send the individual to a nearby urgent care facility for a tetanus shot.

Clearance from the health professional must be returned before access to the animal facility and inclusion on any animal care protocol.

Accommodations for health-related reasons are stated on the clearance form and the Safety Office works with the individual to resolve. Personnel with changes in their health status (e.g. pregnancy) work through the Safety Office to determine if additional precautions or protections are necessary.

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.

Contracted Security personnel are on site 24/7 and will call Safety Office personnel or 911 depending on the nature of the emergency. The site safety plan describes medical services availability. Security personnel have access to the safety plan and safety data sheet (SDS) information for chemical spills.

WVU Medicine at JW Ruby Memorial Hospital is adjacent to the NIOSH facility and is one of five regional medical centers. WVU hospital contains the Jon Michael Moore Trauma Center, a Level 1 trauma center, and the WVU Occupational Medicine Department within the School of Public Health is available to Emergency Responders and is used for foreign travel vaccination requirements. We have an agreement with WVU Medicine Occupational Health clinic for bloodborne pathogen incidents.

Within a mile on the north side of the facility is Mon Health Medical Center with a Level IV trauma center as well as two urgent care facilities (Med Express and WVU Medicine Urgent Care) within a half mile. Employees injured on the job may visit their provider of choice.
The Safety Office also provides notification when CPR classes and refresher courses are available to NIOSH personnel and contractors. All Commissioned Corp staff are certified and a list of additional trained personnel are provided in AED cabinets.
Safety Office personnel, including an Industrial Hygienist, are intimately involved with disaster planning, the Occupational Health Program, safety training, accident reporting and are members of the IACUC which keeps them engaged in animal research program issues.

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.

All animal users beginning in 2015 are assigned the A.L.L. module on *Laboratory Animal Allergy* which provides information relevant to animal work

at NIOSH. Based on the source of animals and species used at NIOSH, zoonotic disease risk is low to non-existent. Personal hygiene and accident reporting are covered in safety training for new employees and are also covered in the site safety plan. Safety training is provided both online and in classes by Safety Office personnel. All employees are required to complete new employee safety training before receiving their smart card and access badge.
The NIOSH Safety Office provides safety training tailored to the needs of housekeeping staff which are contractors. This group has limited access to the laboratories for routine, non-hazardous housekeeping activities.

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.

Entry into the animal facility requires donning of shoe covers by all.
Animal care and research personnel are provided with laundered scrubs for protection of personnel as well as for biosecurity of the animals. Scrubs are not to be worn outside of the building. Animal care personnel are required to wear a hair bouffant, gloves and surgical mask prior to performing husbandry in the animal rooms. Additional PPE in the form of front button/cuffed lab coat or coverall and respirators are available based on risk assessment for the animal colony or personal exposure to experimental agents or existing allergies. Safety shoes are provided to animal care staff by the contractor and are replaced annually.
Research and technical staff that enter animal rooms must wear scrubs and additional PPE as described for animal care personnel above. The exception is for animal rooms in the Inhalation Facility where Inhalation and research technicians may don a full Tyvek coverall over street clothes. All personnel are required to wear shoe covers, a surgical mask and bouffant when entering an animal room.

Researchers are required to wear a buttoned lab coat or scrubs and safety glasses while actively working at the laboratory benchtop, fume hood or biological safety cabinet.
Additional PPE may be required depending on the type of work and the chemicals or agents being used.

b) Describe arrangements for laundering work clothing.

Scrubs and towels from the animal facility and cloth lab coats from research labs are picked up by a commercial laundry vendor (All Season Linen and Mat Service) and returned in protective bags. Scrubs for the animal facility are sorted and either stored in a closed cabinet or autoclaved for use in some animal breeding or surgery rooms.

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.

Sinks with hand soap are provided in certain animal rooms, the break room, cage wash dirty/clean and locker rooms within the animal facility. All personnel are expected to wash hands prior to leaving the animal facility and before eating or drinking. Basic laboratory safety hygiene is covered in various safety training courses. A shower and locker rooms are available for animal care and research staff. Animal care staff must shower and change into clean scrubs if they need to back track from 'dirty' to 'clean' animal use areas. Additional shower facilities are available in the fitness center in the basement.
Scrubs are not permitted to be worn outside of the building. Animal care staff are instructed not to visit public and administrative areas of the building in scrubs worn into animal use areas. Research staff are required to leave scrubs in the facility when they exit.

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

Smoking is not permitted in any part of the building. Smoking shelters are provided outdoors near the parking areas. Eating and drinking is not permitted in any area of the animal facility except the designated breakroom and supervisor office.

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).

As a rodent facility, excessive noise has not been a concern. The Safety Office has monitored noise levels in the cage wash area and ear protection is not required but is available. Sound absorption panels are installed on the walls and ceiling of the cage wash area, and were also recently installed inside machine enclosure shields. Cage wash chemicals are purchased in large drums, and dollies intended for transport of chemical drums are utilized to bring chemicals from the loading dock to the animal facility via a freight elevator.

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

Access to the animal facility and individual animal rooms is restricted by card access/badge readers. Rodent allergens are reduced primarily by engineered methods of work flow. Ventilated caging, purchase of and increased use of cage change stations and/or exhausted work stations (chemical fume hood) and down draft dirty bedding dump stations minimize exposure. Personnel that are identified as needing accommodation based on health reasons can be fit tested for a respirator to reduce inhaled allergens. Scrubs, head covers and gloves must be worn into animal rooms in the core animal facility and coveralls, head covers and gloves are required to be worn over street clothes in animal rooms in the inhalation section of the animal facility. Scrubs are removed prior to leaving the building and disposable PPE are removed upon leaving the animal room. Showering facilities are available to individuals needing or wanting to minimize allergen exposure before leaving the building.

Multiple down draft work stations are available to investigators to use for necropsy in the core pathology laboratory.

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

All rodents are purchased from commercial vendors that surveil colony health and institute practices to prevent entry of rodent zoonotic diseases. Rodents obtained from non-commercial sources are routinely sent directly to a commercial vendor for rederivation and follow up testing which includes common rodent zoonotic diseases. The rodent health surveillance program provides for quarterly testing to monitor for common rodent pathogens. Limited access, required PPE and polices which do not allow animal re-entry or entry by personnel with rodent contact outside of work are practices intended to minimize the risk of entry of zoonotic or other rodent pathogens. The increasing use of cage change stations and the use of exhausted dirty bedding disposal stations minimizes exposure to rodent sourced pathogens.

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

Ventilated equipment such as biosafety cabinets, chemical fume hoods, cage change stations, dirty bedding dump stations, HEPA filtered air supply units and exhaust units on ventilated racks are certified annually by an outside contractor. All units are labeled with the certification date. Any units that are substantially moved (e.g. outside of the AF, stored offsite or newly arrived) are thoroughly washed and recertified prior to use.
Respirators that are not disposable are required to be sanitized after use and inspected prior to use by the user. At the time of annual fit test, the Safety Officer who performs the test inspects the unit and reviews maintenance procedures.

e) Respiratory Protection

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

Respiratory protective equipment is rarely required for the animal research programs at NIOSH. The Safety Office makes a determination that respiratory protection is necessary based on IACUC protocol review. Respiratory protection is focused on engineering controls when possible. All the ACTs are enrolled in the respiratory protection program and have been issued respirators with vapor filters for fogging (decontaminating) rooms and other potential respiratory hazards. A surgical face mask is required when working in the dirty cage wash area. A Class 1 animal bedding disposal cabinet minimizes workers' exposure to dust and allergens when dumping dirty cages.

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

The Safety Office is responsible for the respiratory protection program for the entire site. Once an individual is identified through health history or exposure risk as needing a respirator, they will be given a health assessment questionnaire from the Safety Office to submit to the Occupational Health Medical contractor for evaluation. The Occupational Health Medical contractor will notify the Safety Office if the employee has been cleared for respirator use or requires further medical testing. The employee will be enrolled in the CDC/OSHE/ Morgantown Respiratory Protection Program and required to complete respiratory protection training. Once training has been successfully completed, the employee will be quantitatively fit-tested with a specific respirator (half mask, full face, N95, etc.) and provided with the appropriate respirator cartridges as well as instruction in proper donning, doffing, and maintenance, and storage. Annual training and fit-testing are required as part of the program. Personnel who are not

required to be in the respiratory protection program are still permitted to wear an N95 mask without fit testing.

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

Personnel from the Safety Office are members of the IACUC and review all protocols. The Safety Office also receives the medical clearance for all personnel that may require accommodation including use of respiratory protection. They direct personnel on when respirators are required and what kind of respirator is required (disposable/half face, N/R/P, 95/99/100, etc.) based on potential exposure.
Respirator fit tests are conducted annually by Safety Office personnel with reminders generated by the Safety Training Database. During fit test sessions, review is conducted with personnel regarding equipment maintenance, and quantitative fit testing is performed and documented.

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).

Rack washer
Tunnel washer
Autoclave
Dirty bedding dump stations/disposal cabinets
Training is provided by the Animal Facility Supervisor to animal care staff who operate the equipment. The chemical vendor (Pharmacal) also provides training as needed when changes are made. Informational signage is reviewed during semi-annual IACUC inspection to determine if it is compliant with safety concerns outlined in the Guide. A

preventive maintenance contract for the washers and autoclave is in place and performed quarterly.

ii) List other heavy equipment such as scrapers, tractors, and farm machinery (manufacturer name, model numbers, etc. are not necessary). Describe training programs, informational signage, and other program policies designed to ensure personnel safety when working with such equipment.
Note: If preferred, this information may be provided in a Table or additional Appendix.

Not applicable

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

Not applicable.

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

The Safety Office provides training and guidance on the use of pressurized gas cylinders during routine safety training. Cylinders must be transported with covers in place using hand trucks designed for this use. When in use, cylinders must be secured in a manner approved by the Safety Office. Anesthetic gas (isoflurane) must be used in a chemical fume hood or must be used with a Safety Office-approved exhausted work station. The Safety Office conducts sampling of each station to ensure personnel exposure is minimized.

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.

See Appendix #21 No BSL3/4 agents in animals No select agents in animals No human-origin tissues in animals

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

See Appendix #21

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

Radiation: H^3-thymidine Vibration

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.

Two members of the Safety Office are (member and alternate) members of the IACUC and review all protocols for safety concerns. The regular member is an Industrial Hygienist by education and training. PIs are required to address all safety concerns prior to protocol approval. This would include

ensuring appropriate engineering controls are in place such as requirements to utilize chemical fume hood for dosing animals, administrative controls such as written operating procedures, and lastly appropriate selection of PPE. The AV also reviews all protocols and works with Safety Office to identify risks related to animal husbandry procedures following experimental procedures to ensure the safety of animal care personnel.

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.

1. How are hazards assessed
New processes and procedures are reviewed to assure compliance and adherence to safety policies and procedures.

2. How are procedures developed to manage risks
Job hazard analysis and procedure reviews are utilized to assure compliance with regulatory and facility policies and procedures.

3. Which individuals in Safety Office are responsible
(b)(6)

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

The hazardous waste disposal program is operated out of the Safety Office. All chemical waste is documented and disposed of by Safety Office personnel. The user contacts the Safety Office, the Safety Officer picks up the waste and completes paperwork and chemical identification. The waste is stored in an onsite hazardous waste accumulation area until pick up by an authorized contractor (Chemtron).
Infectious waste generated by the laboratory, or in some cases animal bedding, is orange bagged and must be decontaminated/deactivated by

autoclaving if possible prior to disposal through the municipal waste stream (Republic Services). If for some reason, the material cannot be autoclaved, it is red bagged and boxed for disposal as medical waste through an outside approved contractor. Autoclave cycle logs and standardized procedures based on type of waste and an effective cycle are provided for each autoclave. A member of the Safety Office performs regular checks on the autoclave using a *Bacillus stearothermophilus* -based test as required by the WV Medical Infectious Waste rule 64CSR56.
Sharps such as needles and blades are disposed of in commercial sharps containers or other container approved by the Safety Office. Recapping is not permitted. Sharps containers are picked up by the Safety Office and disposed of through our Medical Infectious Waste contractor. Glass is disposed of in cardboard boxes.
Unfixed animal carcasses/tissues are placed in zippered bags and stored in a dedicated freezer until a scheduled visit by an outside contractor (Stericycle). The animal facility supervisor red bags the carcasses and delivers them to the Safety Officer where they are boxed for pick up.
Fixed tissue (e.g. formalin) is picked up by the Safety Office and disposed of as hazardous waste.
Radioactive waste and a small number of animal carcasses dosed with H^3-thymidine are collected by a Safety Officer, stored in the radioactive waste storage area and disposed of by a radioactive waste disposal contractor (Chase Environmental).

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

Risks for select groups of people such as immunosuppressed or pregnancy are identified during protocol review and additional pre-cautions are documented. These warnings must be posted on the animal room door where the work is being performed. OSHP questionnaires are updated every 5 years.

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.

Upon hire or change in job duties, a Health and Safety Training Checklist is completed by the supervisor to indicate what safety training is required. The Safety Office provides training classes throughout the year to ensure personnel stay up to date. Topics include Safety Orientation for New Hires, Hazardous Communication, Bio-Safety Training, Blood borne Pathogens, Chemical Storage and Handling, Radiation Training and Respiratory Protection. Agents are evaluated by the Safety Officer/IACUC member based on a variety of resources including safety data sheets, internet searches for best practices by other institutions, review of the current literature if there is concern about active excretion by dosed animals and the most current version of the CDC BMBL. This information is documented in the IACUC protocol along with appropriate practices for protection for laboratory and animal facility personnel that work with animals. Personnel are also trained and instructed on appropriate handling methods of animals to minimize risk of exposure to hazardous agents during dosing.

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.

Most animal studies at NIOSH fall within the BSL-1/ABSL-1 category which require basic controls such as limited access to animal housing and procedure rooms. Animals are housed in the Animal Facility core or adjacent Inhalation Facility. Standard/routine PPE is described elsewhere. Some animal rooms contain chemical fume hoods to dose animals with potentially hazardous agents from the workplace. The Safety Office determines the potential for hazards once the animals are dosed so that precautions including soiled bedding disposal are addressed. Non-hazardous animal bedding is disposed

of as landfill waste. Procedure and animal rooms in the inhalation part of the facility are negative to the corridor. Dirty cage wash areas, procedure and animal rooms in the animal facility are negative to the corridor. Animal breeding rooms and the surgery room in the animal facility are positive to the corridor. BSL-2 agents described elsewhere are conducted in a dedicated room to allow for additional restriction on access, negative room pressure, biosafety cabinet, PPE and dedicated equipment that cannot be removed or shared without appropriate decontamination. Additional requirements for working in this room including animal waste disposal and cage sanitation are addressed in the special concerns for animal use section of the approved protocol.

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.

The one animal housing room outside the core facility, (b)(7)(E), is a standard housing room with standard procedures employed. Being a satellite room, it is regarded as less clean and animals are not permitted to return from it to the core facility. An exception to that rule is if it is employed for quarantine procedures, in which case it will be operated under strict containment with negative room pressure. Recent use of this room has been for experimental mice used in a muscle strength, conditioning & atrophy study and for rats in a vibration study; therefore, additional measures for hazard containment have not been necessary.

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

All ventilated safety equipment is certified annually and must be recertified if moved. Equipment includes Class II biosafety cabinets, chemical fume hoods in animal rooms and inhalation facility, cage change stations, ventilated cage racks with HEPA filtered air supply units, and soiled bedding dump stations.

Most of the isoflurane vaporizer stations are equipped with a downdraft exhaust platform to remove gas away from the operator, and isoflurane used in bell jar type set ups is required to be used in a chemical fume hood.

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

During protocol and amendment review, the safety personnel on the IACUC provide input on what additional PPE, administrative and engineering controls are required to conduct animal studies. Personnel working with animals in the animal facility core are required to wear scrubs which are laundered through a contractor. Hair bouffant and gloves are also required. Additional protection may be required, such as protective sleeves or a lab coat/gown for dermal hazards. Signage is added to rooms and cages if there are risk to specific groups (e.g. pregnant or immunocompromised) or for specific hazardous agents that pose a risk after the animals are treated. Most soiled cages are changed in ventilated workstations and soiled bedding is dumped into a ventilated dump station in the dirty side of the cage wash.

e) Incidental Animal Contact and Patient Areas

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

Not applicable

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

Experimental animals are transported from the AF to laboratories for final disposition. Animals are secured in a filter topped micro-isolator cage.

The transport cart is then covered by a drape to reduce animal stress. Animals may only be transported on the freight elevators. The animals are delivered to the hallway outside of the laboratory, and research personnel pull the animals into the laboratory. The laboratory areas are restricted access, and entry requires badge access. The doors are marked to indicate when animals are present. One exception is animals that are housed in (b)(7)(E) may leave (to labs for experiments) and return; however, the transport requirements are the same. Empty cages are covered and returned to the AF for processing in the dirty side cage wash.

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.

Recommendations are made to the HELD Director by the IACUC Administrative Group. Candidate names chosen by the HELD Director are forwarded to the IO who makes the appointment and responds with a letter of appointment which is maintained in the IACUC files. New members are usually appointed as alternate members to allow for training, experience and mentoring. No more than three full members, including the Chair, are appointed from each Branch.

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

The IACUC has scheduled meetings the 3rd Thursday of each month. The schedule is posted on the HELD announcement screen. Some shifting of monthly meetings may occur due to holidays or

during Society of Toxicology meetings, which are heavily attended by IACUC members. Semi-annual program review takes place at the April and October meetings. Additional meetings are scheduled as needed.

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

New members are generally appointed as alternates to allow for training, experience and mentoring. The Administrative Group meets with new members to review training materials and assignments and discuss the animal care and use program from the IACUC standpoint. Meeting attendance expectations, conflict of interest, confidentiality, and protocol review are also covered. The A.L.L. module *Working with the IACUC* is assigned and other relevant modules that may be of interest are pointed out. New members are assigned as secondary reviewers to gain experience in review. Full and alternate members attend monthly meetings to keep up with current issues and programmatic changes.
When funding is available, additional outside training is supported by the HELD OD. OLAW, AALAS and PRIMR webinars are shared with the committee when appropriate. Lab Animal journal IACUC cases are sometimes shared during committee meetings when the topic is relevant to the NIOSH program.

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 new protocols are reviewed at a convened meeting of the IACUC. At the end of a 3 year period, the PI must submit a new protocol as this helps to ensure the protocol is consistent with updated policies and minimizes protocol drift. Protocols undergo an annual review in October. *De novo* protocols are submitted through Topaz Elements software program by the PI. The IA forwards the protocol for a Tier I review by the IA, AV and Safety Office. Once complete, the protocol may be returned to the PI for revisions or if there are no substantial issues to be resolved the IA forwards the protocol to the entire IACUC for a Tier II-FCR for which the Chair assigns a Primary and Secondary reviewer. All new protocols go to FCR. The primary and secondary reviewer summarize review comments and present the protocol at the convened meeting (of a quorum) with their recommendation for action. The members or assigned alternate vote to approve, return for modification followed by DMR (Primary as assigned by the Chair), return for modification followed by return to FCR, or withhold approval. NOTE: The IACUC has a unanimously approved policy that is reviewed annually that allows a quorum of a convened meeting to vote to send protocols from FCR to DMR. The vote must be unanimous to defer to DMR.

The IACUC uses the USDA pain categories to classify all vertebrate animal use. Discussions during convened meetings include; if the animals have been categorized correctly, has the PI provided in the case of category E animals a sufficient description of time course and clinical signs and if not will PAM be required to complete a full assessment and develop humane endpoints with input and approval by the AV. Has the PI conveyed the importance of the study and do the members understand. The alternatives search terms are evaluated to determine if they are appropriate and likely to identify the 3R's. A separate section of the protocol requires the PI to address species selection and duplication. The form requires the PI to provide the strategy for how they determined unnecessary duplication. HELD has biostatistical consultation service in BB for HELD scientists and most PIs take advantage of this service.

July 2020- Following COVID period guidance put out by OLAW in April, our IACUC amended our policy on protocol review (P-0006) to extend expiring protocols for up to six months by undergoing another full committee review (FCR), at the request of the PI. We also began offering PIs delayed

approval of original protocols that have gone through FCR +/- subsequent designated member review (DMR) successfully. Delayed approval is indefinite until NIOSH reopens or at the request of the PI.

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.

Modifications to protocols are called amendments. We use Topaz Elements software to conduct reviews of all amendment and protocols. Amendments are sent to all committee members, who have 48 hours to review and call for FCR. If there are no calls for FCR the Chair assigns at least one qualified reviewer, and the protocol amendment enters the DMR process after the 48 hours have passed. By default, the AV and the Safety Officer are part of the DMR group. The result of DMR review can be to return the document for modification to obtain approval, or outright approval. The group opinion must be unanimous, or the amendment is steered to the FCR process described above. If returned for modification, the protocol is returned to the previously assigned DMR group to re-review under DMR, however there is no requirement for re-review by all members for subsequent revision of the same amendment.
Modifications that can be handled by Administrative action by the IA, who is a voting member of the IACUC, are grammatical or spelling errors and addition of personnel to the protocol other than a change in the PI. Modifications that can be handled by VVC include minor procedural changes, an increase in the number of animals up to 10%, and technical changes in drugs used; these are detailed in the updated IACUC Policy-0006. Modifications are considered significant/major as defined by NOT-OD-14-126.

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.

Humane endpoints as applied to Category E studies is a separate principle from the AV being provided the authority to euthanize animals in distress which had

not been anticipated in the original study design. Category E studies are defined as pain, distress, permanent dysfunction that may alter the animal's ability to cope with its environment or metabolic disruption that impairs an animal's ability to maintain homeostasis for which analgesia or palliative care cannot be provided for justifiable scientific reasons or are not effective. While the FCR begins with a thorough description by the PI during the review process, the IACUC recognizes the need to observe these animals to ensure the description provided matches what actually occurs. PAM is frequently utilized for such purposes on Category E studies. The animal care staff is helpful in this regard for reporting concerns or timelines that are not documented. For new agents or for studies where category E has been determined after the beginning of an approved study, a more extensive and frequent observation record is utilized with input/design by the AV. Once data is obtained, it is reviewed by the research team, AV and IACUC, and an attempt is made to develop a scoring system or a predictive sign that can be used to determine when to provide an early intervention (e.g. euthanasia for humane reasons). One investigator has a number of protocols with a large proportion of Category E animals to study effects and treatment of nerve agent exposure for the Gulf War Illness syndrome in soldiers. Acetylcholinesterase inhibitors used cause substantial morbidity and mortality; for all these studies the IACUC and investigators have a 48-hour post-exposure rubric for twice daily clinical scoring and endpoint determination.

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).

The IACUC is dedicated to employing early endpoints when unalleviated pain or distress is anticipated in a study; toward that end subcommittee meetings are held with PIs to ascertain their experimental data requirements and refine their study to minimize the incidence and duration of pain and distress. The pain or distress may be self-identified by the PI or through the review process. If there is insufficient data available from previous studies or the literature to determine

the time course (onset and duration) of pain/distress, moribundity or mortality, the IACUC may require a pilot study using less animals. During the pilot work, the AV and committee members will observe animals with the research staff and review observation logs to better understand how the animal responds. Based on data and observation, humane endpoints are developed, and palliative care considered. The committee also depends on PAM by the AV and IACUC members to refine category E studies as an ongoing process. Observations by the animal care staff are also important, and animal health reports generated in Topaz are sent to the AV and the PI. The PI may also be required to submit additional reports to determine the accuracy of the humane endpoint plan that is approved.

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.

Research staff on approved protocols, animal care staff and the AV are all responsible for monitoring the welfare of research animals. For studies identified during protocol review that are likely to cause pain/distress the research staff has primary responsibility for providing a plan to monitor animals to include what to look for in addition to the frequency and duration of the observation periods. PIs are responsible for training new personnel; however, the A.L.L. species-specific modules are also available that cover these topics. Introductory training is also available during orientation to the animal facility for new employees.
The animal care staff are encouraged to report concerns regardless of 'approved procedures' as animals may respond differently than expected. At least half the staff have over 8 years' experience which aids in the mentoring process of new hires. During animal care staff meetings there are discussions on how nesting and other behavior can be used to assess welfare in mice, how animals behave in response to pain such as chewing at incision/ injection sites, increased aggression when handled (geriatric mice), and behavioral differences in different strains/stocks/genetically modified animals. The AV frequently meets with research and animal facility personnel to observe animals together to

ensure personnel understand what they are observing and are able to accurately report what is being seen. These principles must come first so that the interpretation that follows is accurate. For routine health reporting, the animal caretakers use a web-based animal health report form, which requires documentation of findings before an interpretation is made. This has helped to ensure continuity of care and allow different observers to determine if the animal is improving or declining.
The phone list posted in the animal facility also provides access to the AV if questions regarding health and welfare arise while personnel are working in the animal facility.

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.

Phenotypes of genetically modified animals which may predispose them to morbidity are identified 1) by PI-provided information, 2) AV review of the vendor website for issues related to welfare and husbandry, and 3) review of the literature. These issues are discussed during protocol review and, if necessary, a plan to provide appropriate support is included in the protocol. However, some genetically modified animals are described in a single paper or the animals are cryopreserved and there is not much data to provide sufficient information on animal needs. In these cases, the animal care staff is tasked with reporting concerns. In situations unrelated to genetically modified animals, both the research and animal care staff are responsible for reporting adverse events and adverse effects that were not documented in the approved protocol to the AV and IACUC in a timely manner. During review, the IACUC frequently asks the PI to provide background on how dose was determined. However, published information is not always complete and information like LD50 data is inaccurate for a variety of reasons. In some cases where welfare is more of a concern due to lack of information, the IACUC will insist on pilot studies to determine dose and/or effects. With this information, humane endpoints and/or improved monitoring plans can be developed. Once a protocol is approved, we ask the PI to fill out a Special Care and Instruction form which summarizes for the care staff any restrictions on diet, enrichment or treatment for animals that may become ill. Also in the past year, we have instituted a new protocol Animal Facility Support Meeting that brings the research staff, AV, AFS and pertinent animal care technicians together to coordinate animal breeding

or ordering and husbandry, and to discuss phenotypic or experimental related health problems that may be seen, as well as support requirements or restrictions.

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."

The NIOSH IACUC has made a distinction between restraint and confinement. In confinement the animal is restricted to a space less than the minimum for housing described in the Guide (or AWRs for guinea pigs), however the animal can still stand on all fours, turn around, lay down and perform normal postural movements. In addition, the time in this environment is much less than 12 hours and is generally reserved for whole body inhalation and non-invasive plethysmograph studies.

Both rats and mice in confinement cages have tactile contact with conspecifics and have been observed by multiple IACUC members. The animals are generally found sleeping during these exposure periods, and no clinical signs of distress have been observed.

Past studies have utilized commercially available metabolic cages to collect urine from rats. The IACUC considers these animals confined, not restrained. The animals are housed in these cages for the minimum time required to collect adequate samples and are returned to their home cages between collection periods. Animals are supplied with food and water in the metabolic cages and have the ability to turn around, lie down and make postural adjustments.

Restraint is considered a space restriction that does not allow the animal to turn around and prevents many normal postural adjustments, and the period of time is less than 30 minutes; therefore, rodent restraint used for sample collection or minor procedures such as tail tattooing are not considered prolonged restraint by the NIOSH IACUC.

July 2020- Policy (P-0010) definition of prolonged restraint is defined as the physical immobilization of animal(s) for periods greater than 30 minutes.

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.

Restraint tubes are used for rodents undergoing nose-only inhalation studies, in which they are restrained for periods less than 2 hours, which includes the loading and unloading time. There are two different systems to administer compounds by this route, one for mice and one for rats. PIs typically utilize a nose-only exposure system when the experimental agent is limited in supply or there are valid concerns regarding an ingested dose if the animal grooms. In the mouse system (in (b)(7)(E) Inhalation Facility), opaque tubing of similar diameter to the restraint tube is supplied in the animal's cage at least a week in advance so that they are allowed to explore and pass through. Prior to the first exposure, mice are placed in the nose-only pods and chambers twice: 20 minutes on the first day and 60 minutes on the second day. The mice can be easily visualized in this system during the exposure event (approximately 1 hour) and animals in distress can be removed from study. For the rat system, housed in the (b)(7)(E) lab, animals are placed in the tubes and acclimated to restraint for 30 and 60 minutes on days -5 and -4 prior to exposure, respectively. The exposure is for 60 minutes performed three days in a row, with an option to repeat the regimen approximately two weeks later. Animals are monitored continuously during the acclimation procedure. For either system, animals that fail to acclimate as determined by continuous attempts to

escape or that vocalize are to be removed from the study. Any death, injury or welfare concern during the restraint period or after the animal is removed is reported to the AV, who reports to the IACUC as part of the veterinary report during convened monthly meetings.
A study of vibration in rats, confines rats for 4 hours/day for up to 10 days to apply the vibration treatment. The PI describes a process which involves increasing lengths of times in the Broome type restrainer in the days preceding the study. The PI has agreed to remove animals that do not acclimate as evidenced by continued struggle and/or vocalization.
Restraint for tattooing or tail-nicks for blood collection rarely lasts 5 minutes: For the AIMS tattooing of mice, they are placed in a small vertical cylinder notched with a slot for the tail and restrained by the base of the tail. The Lab Stamp tattooing system for mice comes with a couple of different sizes of Broome style restraint cartridges. Rats are restrained in a Broome type restraint device, or occasionally a Decapicone, or wrapped in a towel with a second person to assist.

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.

There have not been any proposals to perform multiple survival surgeries. However, scientific justification would be required including why an alternative approach, for example use of a non-survival second surgery endpoint, could not be used. Cost savings would not be a sufficient justification. Extra scrutiny would be given to post-operative monitoring, the pain management plan, frequency, and absolute number of surgical events, and risk of permanent physiological impairment of organs or limbs.

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.

Not applicable

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.

There are no NIOSH protocols approved for food or fluid regulation. The closest thing to it are two rat studies in which food, but not water, is withheld overnight to fast the rats prior to their experimental endpoint the following morning. The food is withheld for not more than 16 hours before euthanasia.

vi. Use of 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.

Pharmaceutical compounds are those medical or veterinary products approved by the FDA or CVM and can be found in the Green or Orange book on the FDA website. Pharmaceutical 'drugs' must be used for anesthesia, analgesia and veterinary clinical care. USP, non-pharmaceutical is the second tier of recommended use and is generally used when the formulation of the pharmaceutical is not compatible with injection such as a pharmaceutical compound in a tablet form with binders, or the pharmaceutical compound contains

additives that the PI believes may interfere with research. Many of the non-pharmaceutical compounds used in animals are experimental agents under study for human occupational health, questioning the toxicity of the agent as it exists in the workplace environment, contaminants and all.

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.

Not applicable.

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

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

Animal reuse is not practiced at NIOSH. Excess animals generated by the breeding program are made available for training or as sentinels, but animals are naïve when transferred.

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.

Not applicable.

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.

Not applicable. All animals are euthanized at the end of study.

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).

All protocols are approved for a 3-year cycle with annual review/update. At the end of the 3-year period, protocol renewals must be revised and resubmitted to ensure they are up to date with current practices and policies. The animal care staff is vital to the PAM program since they are the individuals with the most contact with the animals. The staff is encouraged to share concerns of welfare and non-compliance with their supervisor and the AV. The HELD program was able to create a dedicated position for IACUC Administrator (IA) in December 2015, which has enhanced the PAM program oversight through improved coordination and availability to visit with animal users.

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

Semi-annual program reviews are performed and discussed in the April and October IACUC meetings; however, programmatic issues are addressed year round. The program review uses a variety of tools to meet the requirement but also to keep members engaged. Methods that have been used to perform program review include use of the OLAW checklist, review of the program charter, review of the HELD SOP database for active animal-use SOPs, IACUC policies review, and review of the AAALAC program description and the PHS Assurance. Deficiencies as well as suggestions for improvement (SFI) are identified. Following the semi-annual review, a report is constructed, approved and signed by all members. The report is forwarded to the HELD Director and CDC IO.

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**.

The semi-annual inspection of the animal facility and labs is performed by an IACUC Inspection team comprised of at least two voting members in April and October. Two forms are provided for the inspection, one for the animal facility and the other for labs. The inspection forms for the laboratories and animal facility are sent to the investigators, Attending Veterinarian and Animal Facility Supervisor one week in advance of the scheduled inspections; they are requested to fill out the forms and return to the IACUC prior to the lab inspection. The IACUC inspection team reviews these forms prior to visiting the labs and follows up on the information provided by the investigators/AV/ AFS during the inspection. Deficiencies are identified and shared with responsible parties and to obtain reasonable dates for correction. The findings are discussed at an IACUC meeting to determine if the deficiency is significant (danger to human or animal health) or minor and if the date for correction is acceptable. A report is prepared, signed off by the IACUC and submitted to the HELD Director and CDC IO. A list of outstanding actions is maintained by the IA and remain on the monthly agenda until resolved.

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.

As a federal facility, USDA does not routinely inspect the NIOSH animal care program. OLAW performed a site visit outside the scope of this time period.

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

During the Division process of publication clearance, documents such as journal submissions or meeting abstracts are reviewed by the Division leadership and the AV or IA. The IA or AV reviews the documents for agreement between submissions and IACUC approved protocols. The IACUC Chair provides backup if the AV is unavailable.

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

The mechanism for reporting animal welfare concerns is multi-pronged. Animal users, security personnel, and visitors are made aware of the animal care and welfare policies and procedures by completing the Animal User (for new employees) or Visitor Agreement Forms, which they must sign. These agreements state that a reporting mechanism exists if they have concerns regarding the welfare or use of animals at NIOSH. Division staff meetings, known as 'All Hands Meetings,' are held quarterly and serve as an additional venue where information is shared with animal users on topics such as IACUC policies and compliance issues. Colorful signs noting IACUC policy and various reporting mechanisms (i.e., direct, indirect, or anonymous) are posted in the animal facility and laboratory hallways. Updated signage is supplied to all laboratories during the semi-annual inspections, and old signage is collected for disposal. The policy includes verbiage noting whistleblower protection for anyone filing a concern/report.
Concerns received are referred to the Chair, or Vice-Chair if there is a conflict of interest. Concerns are discussed at a convened quorum of an IACUC meeting. If an investigation is deemed necessary, the Chair appoints at least two IACUC members to conduct interviews and observe, collect, record any physical evidence associated with the case. The Chair may initiate an investigation prior to a convened IACUC meeting if significant animal welfare concerns exist. If the initial report and findings suggest a non-compliance reportable to OLAW, the IO is notified by phone or email that an investigation is underway. The findings are presented at a convened meeting, which the Chair may call outside of the normal monthly meeting schedule to determine if the findings rise to the level of protocol non-compliance and/or a significant or minor programmatic deficiency using The Guide and AWR for reference. A report is generated to the HELD Director and forwarded to the IO, if it is determined there has been a protocol non-compliance or significant program issue and includes the plan for resolution and the deadline for correction. The process is usually accomplished within 60 days from the incident.

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.

There is a site wide disaster plan which includes specific plans for the animal facility and procedure areas outside of the AF core where animals might be found during an emergency. The plans are available on the shared drive for NIOSH personnel access, and there is a hard copy at the Security desk. There is an emergency response team (ERT) which is outlined in the disaster plan by name and function, and the AV is a part of that team with responsibilities for the animals and animal facility.
The plan outlines the procedures for triage, animals under anesthesia during evacuations, securing animals left behind (shelter in place), euthanasia methods outside of protocol approval, and notification of the AV for follow up during and following an emergency. There are also checklists included in the plan to manage emergency care of animals with minimal resources.
July 2020- Newly updated/ approved Animal Emergency Response Plan (AERP) and appendices. The two appendices (one a checklist) are required reading for all investigators and are maintained by the PI in each laboratory inside their ACUP binder.

II. Animal Environment, Housing and Management

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

A. Animal Environment

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

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

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

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

All animal housing areas are monitored at least daily by animal care personnel (or a member of the research staff if housed outside of the animal facility). In addition to animal welfare checks, the temperature and humidity are observed and documented in the room log by checking a thermohygrometer placed in each room. Values outside of the room normal range are reported to the Animal Facility Supervisor who reports and follows up with OFM personnel to correct the problem. Values that are outside of the primary range and may affect animal health and welfare are reported to the AV as well. Animal housing rooms are also monitored by an automated building control system which will alarm at the security desk if room temperature goes out of range. During regular working hours, OFM is notified and corrects the problem. Outside of normal working hours, security personnel contact OFM, the AV and AF Supervisor about excursions. Depending on the area, the degree of excursion or the ability to correct in a timely manner, the AV and AF Supervisor will return to the building to check on the animals or will make arrangements to move the animals to a different location.

July 2020- OFM is now FMO (Facilities Management Office)

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]

Mice, Rats and Guinea pigs
Set point – 72F +/- 2F
Report if <69F or >76F or relative humidity very far outside of 30-70% range

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]

All animals are housed with contact bedding in the cage. Based on the current literature regarding cold stress in mice, all mice receive nesting material. However, a PI could request an exemption provided they address the cold stress issue as it relates to their study. Social housing is the default arrangement, and most mice and rats sleep and rest in a pile. Guinea pigs, some rats and mice may be provided structural shelters (igloos, tubes and huts) which also provide the animal the ability to nest.

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.

Supply air (100% fresh) to the entire central Animal Facility is HEPA filtered to remove >90% of particles larger than 1 micron. Room ventilation is controlled and monitored by the building's computer-controlled management system (being upgraded to Distech Controls), recorded and reported in the same way as temperature and humidity. Room ventilation rate is set at 10 ACH or higher. Each room exhaust is controlled by a continuously variable Phoenix venturi valve. There is an ongoing renovation of control valves and sensors for laboratories and animal rooms, with the core animal facility scheduled for 2020. Monitors outside of each room have been installed that report each room's pressure gradient to the building monitoring system. Rooms in the AF are evaluated when room function changes. When these parameters are out of range, the system alarms to the security desk and OFM is contacted. Outside of normal working hours, the AV and AF Supervisor are also contacted. Reports can be generated if problems persist. Ventilation rates are evaluated every 3 years.

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

With few exceptions, all rodent caging is individually ventilated caging (IVC) using filter top cages on ventilated racks. Supply and some exhaust air is HEPA filtered. IVC supply air comes from the room, and exhaust air may be exhausted to the room or ducted into the building exhaust. Pre-filters are changed on a monthly schedule or as

required by increased resistance detected by magnehelic gauges, which are monitored by AF staff. IVC racks are set to run positive to the room.
Exceptions to ventilated caging (i.e. static caging) are due to the need to collect data from instrumented animals where data collection equipment is not compatible with the caging/rack systems.

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.

No air is recycled in the core animal facility, the SAF or laboratories.

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

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

Not applicable.

b. Provide a general description of overall system(s) design, housing densities, and water treatment, maintenance, and quality assurance that are used to ensure species appropriateness.
Note: Facility-specific tank design and parameter monitoring frequencies should be summarized in **Appendix 12** (Aquatic Systems Summary).

Not applicable.

4. Noise and [*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.

The NIOSH animal facility is a rodent only facility and is therefore relatively quiet. Animal care staff and research staff during orientation are apprised of the need to keep noise to a minimum. At least a third of the animal census is comprised of breeding mice

with generally excellent reproduction results which also is a reflection of minimal noise and vibration that might have a negative influence on breeding. Our main concern with noise and vibration is with the ongoing building façade repair which began in September 2019. Sound meters and vibration recorders have been used to periodically monitor the situation and respond appropriately.
Animal care technicians conduct regular maintenance of rack fan motors and will replace units where vibration or unusual noise is detected.

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 most recent version of the PHS Guide for Care and Use of Laboratory Animals is the primary reference used to determine minimal husbandry requirements for rodents. The Animal Welfare regulations are used when guinea pigs are on site. While minimum standards are met for housing, social housing takes precedence and animal care personnel will change cages more frequently if larger phenotype animals are observed in soiled or wet cages.
Many studies routinely weigh animals and can provide notice when group housed animals approach limits. Density guidelines are posted in the animal rooms, and animal care staff will weigh animals if it appears that animals are reaching the limits provided. Sprague Dawley rats that are maintained past six months of age get quite large and in order to socially house these animals, Lab Products caging was purchased to provide additional space and is now our standard rat caging. All AF animal rooms are inspected by members of the IACUC during semi-annual inspection; the rooms are entered and animals observed in their home cages.

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]

There is an AF guideline for cage density for rodents which is based on the minimum standards found in the Guide.

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).

Caging floor space and height meet the minimum requirements outlined in *The Guide* which in most cases allow rats and mice the ability to move about the floor and rear up on their hind limbs. When cage space is adequate and the addition will not interfere with scientific design, shelves and shelters may be added to the cages of mice and rats to allow them to climb and shelter/nest. These same devices may not be permitted with some group-housed male mice which can use raised platforms to ambush cage mates. Guinea pigs are provided 'huts' to provide a hide box structure compatible with their behavioral needs.

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).

We have found the most success, based on their use of and quality of nest building, with kraft paper nesting material for mice. A partial change is made at regular cage change as the product quality will diminish after excessive use. For some strains,

the addition of cotton square type nesting material improves nest quality/used. For rats, social housing appears to encourage play behaviors and the animal care staff are encouraged to handle animals to both gentle them and provide enrichment. Nylabones are provided to rats to gnaw when other manipulanda is not permitted. For breeding animals, food treats are used to provide enrichment as well as improve production. In the past year the use of wooden blocks and gnawing sticks (manzanita) have been employed as a means of dental maintenance for malocclusion and as a diversion from antisocial behavior or food grinding.

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

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

The default housing arrangement for rodents is social. In recent years we have had good success with group housing male mice up to nine months of age by ordering them at weaning age and providing nesting material. We also wean into experimental groups so the animals are in established social groups upon receipt. We strive to maintain a group size of 2-5 in standard shoebox sized cages for mice, and once the animals have acclimated after arrival do not permit regrouping unless specifically described and justified in the protocol. We tolerate some tail biting if the wounding is not severe and repeated. Since scientific evidence suggests that barbering is not a behavior based on aggression, we leave animals in their social group when barbering is reported as long as there are no skin lesions.
For rats we primarily pair house although density is an issue with older animals on a Sprague Dawley background. As long as the animals stay clean and dry and have space to move around the cage, we will leave them paired.
Sentinel animals are pair housed unless one of the pair dies during their tenure.

July 2020- For clarification, guinea pigs were housed here since the 2017 AAALAC site visit, but we've had none in the facility for over two years

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 animal care and use protocol contains a question specific to social housing. The PI must provide scientific justification for single housing rodents. Since we have found that male mice can be grouped in certain situations, the bar is higher for PIs to obtain approval for single housing. Justifiable reasons for single housing include 1) an ongoing study where animals were single housed in previous studies and continuity is required to compare results, 2) the PI provides empirical and relevant data that single and group housing provide different results, 3) severe toxicity that makes it difficult to monitor animals in group housing, 4) cohort design where a single animal undergoes different treatment from other animals at a specific time point, and additional animals cannot be used due to limitation in resources (equipment/personnel), 5) documented severe aggression for specific mouse strains on long term studies, and 6) breeding colony (breeding males, pregnant dams, odd sex at weaning).
Under some circumstances the veterinarian may request animals be separated when animals are acutely injured, or aggression is continuous and concerns are raised regarding serious injury or death. This is not common.

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

Animal care staff are instructed to handle animals gently and positively during cage changes to reduce handling stress to make their job easier as well as improve the lives of the animal. Additional nesting material is provided as well.

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 IACUC has a social housing policy which provides accepted rationales for single housing. Policies are updated as needed and in the future will likely be added to the

same DMR process that is used for HELD SOPs which is review every 2 years per CDC policy. There are currently no studies where mice are housed without nesting material as PIs have been asked to provide justification for cold stress in their studies if nesting material cannot be added to cages. Rats are provided either a structure or Nylabone, often both, depending on study requirements, but pairing appears to be the most enriching.

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.

Our animal care staff has many years' experience with rodents and work very hard to handle each animal on entry gently and quietly with minimal restraint. Rodents are touched and stroked to become familiar with touch. Additional information is sought for mouse strains that are identified as 'aggressive' to determine if they have a reported phenotype for blindness or deafness and if so to be sure to take extra care to let these animals know that they are being approached so they are not startled and respond aggressively. The rat strains we use are fairly affable and we have experimented with rat 'tickling'.

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

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

Not applicable.

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

Not applicable.

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

In social housing environments, structures are not permitted that do not allow escape (e.g. igloos or huts must have at least two openings).

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).

Not applicable.

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

Not applicable.

iii. Describe how animals are captured.

Not applicable.

C. Animal Facility Management

1. Husbandry

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

i. List type and source of food stuffs.

The majority of mice are fed Envigo RMS, Irradiated NIH-31 Modified 6%, rats are fed Envigo RMS, Irradiated Teklad Global 18% and Guinea pigs are fed Teklad Guinea Pig.
Study specific diets include – Envigo Irradiated NIH-31 Open pelletized and meal for Snell dwarf breeding colony and study animals; Irradiated adjusted calorie (45 g

fat) and (60 g fat) for 2 different rat studies; and Zeigler NTP 2000 for NTP mouse studies.
Bio-Serv transgenic dough, love mash and irradiated sunflower seeds are used to improve breeding performance and for enrichment.
See **appendix 20** for a complete list of products used.

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

Vendors are provided above.
Feed and bedding are stored in the same rooms (b)(7)(E) The room temperature in storage rooms is set to 68F, and excursions alarm through an automated system which is monitored by OFM and security personnel. Animal facility staff monitor these rooms daily and report excursions if present. When the temperature or humidity in a room is borderline or questionable, we request a monitoring record of the room from the Facilities Management staff. When the new building monitoring system is fully operational, we will have access to this ourselves, in addition to a real time overview of temperature, RH, and ventilation.
Due to the use of high fat diets, that must be stored at cooler temperatures, walk in cooler space is being shared with ACI Branch. The temperature is set to 4C. As these diets generally have shortened shelf life they can be stored in the refrigerator in the animal room. Feed bags are marked and rotated so that the oldest lots are used first.
The outside surface of each bag is wiped down with Clidox-S or Virkon-S prior to passage through the 'clean' airlock of the animal facility. When food supplies are replenished in the animal room, the contents of the original bag are transferred into

a lined feed storage bin in the animal room. The lot number is recorded in the assigned room log book.
Vermin control in feed/bedding storage areas is consistent with the rest of the facility. Visual observations are made to determine if animals are present, such as the presence of fecal material. Sticky traps are used to collect and identify insects. Live rodent traps may be placed if there is evidence of entry, which historically has not been an issue. Regular housekeeping to prevent the accumulation of spills, and storage of feed and bedding raised off the floor improves the ability to detect incursion.

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

Not applicable.

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

The majority of pelletized diet is delivered by placement in the cage lid hopper or hanging hopper for some rats and guinea pigs. A multiple day supply is provided in the hopper, checked daily and topped off as needed until the scheduled cage change. One study using dwarf mice with a phenotype that does not include full dentition are provided food in a powdered form using commercially available feed jars.
In the breeding colonies, a few pellets or treat type food are placed on the floor for young animals to transition through the first week after weaning. Food treats such as sunflower seeds or 'trans-dough' are placed on the cage floor to improve reproductive performance in some strains of mice.
Rats with head caps were fed on the cage floor and provided Napa nectar as a water substitute to minimize the risk of dislodging the head cap on feeders and sipper tubes.

Animals that are identified with weight loss, poor consumption, malocclusion, new arrival of very young mice, etc., may be offered moistened food pellets until their case is reviewed by the AV to encourage hydration/consumption.
Certain category E studies have a palliative care plan described in the approved protocol which utilizes a mash made from ground food pellets and water placed near animals that are temporarily incapacitated and unable to reach the food hopper or water bottle.

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

Feed bags are inspected on arrival by animal care staff to identify possible contamination by penetration of the primary cover. Broken bags are not used. The outer surface of the bag is wiped with disinfectant before passage through the 'clean' AF air lock. Feed is purchased from reputable vendors and in most cases is irradiated to reduce concerns of contamination during processing. Feed bags are rotated so that earlier lots of food are used first. Milling/manufacture dates are observed and feed is used within 6-9 months of milling. The feed vendor states that small vacuum packed bags of irradiated feed have a shelf life of up to nine months. When feed is taken to an animal room, the lot number is recorded in the room log book. The ascorbic acid in the guinea pig feed is stabilized and allows storage for up to 6 months versus the previous shelf-life of 90 days.

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).

Municipal sourced tap water is the primary drinking water used for research rodents. (b)(4) (b)(4) tests samples from the AF annually for presence of coliforms/ E.coli. Provision of water for all rodents housed in the AF core rooms and SAF is in bottles filled at the bottle filling station in room (b)(4)

A comprehensive testing of water from this point was conducted in 2019 (microbiological, pesticides, heavy metals, asbestos) and all tests met or exceeded EPA standards for drinking water. Since then an Edstrom/Avidity filter bank has been installed in the line to the bottle filler to ensure water purity.
One PI utilizes water from a purification system (RO and ultrafiltration) in his laboratory to provide agent spiked water to a subset of research mice or rats. This water is delivered by water bottle for up to 7 days at a time.
All animals are currently provided water by a bottle on the cage lid. One study used instrumented rats with head caps. These animals were provided Napa Nectar as there was concern that animals would traumatize the surgical site or dislodge the device on the sipper tube or cage lid. The animals did well on the water substitute, and the packaging provided enrichment based on observations of play behavior.

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

The (b)(4) ap water is evaluated by review of the annual (b)(4) water quality report and an annual testing of water sampled from the animal facility bottle filler and three other sites (in the AF, IF and one PI laboratory) for coliforms and E.coli.
One PI utilizes water from a reverse osmosis ultrafiltration system in the laboratory to provide agent spiked water to a subset of research mice or rats. A comprehensive testing of water sampled from the bottle filler in the Animal Facility was conducted in 2019. This included heavy metals, microbiological, and pesticides; results were all negative or within the EPA guideline limits. Following this an Edstrom/Avidity microfiltration system was installed in the line to the bottle filler in (b)(4)

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

Not applicable

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

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

Most research rats are provided a combination of Envigo Sani-chip and Diamond Dry or Alpha-dri as bedding. Some rats are provided Diamond Dry/Alpha-dri exclusively to monitor animals post procedurally. Sentinel rats are provided the same bedding as the animals they are monitoring, but will on occasion be provided with novel bedding for enrichment and to 'test' different/new bedding products.
We use the Crink l'nest with our breeding rats and while they do not build a nest, they do incorporate the product in their nest area.
Most research mice are provided Envigo Sani-chip with nesting material. Crink l'nest Kraft irradiated nesting material is used almost exclusively as we have observed the most complex nest building using this material. Mice on the Gulf War Illness studies, which are prone to hypothermia following exposure to an acetylcholinesterase inhibitor, are provided a cotton square Nestlet in addition to the Crink l'nest, which has improved their nest building quality.
Guinea pigs are rarely used. Most recently the hairless guinea pigs were housed on a combination of Diamond Dry and Cellu-nest to minimize skin trauma and provide a more absorbent bedding to address their less fastidious behavior. White paper chip bedding (Diamond Dry or Alpha-dri) is available for PIs upon request and frequently recommended by the AV for specific animals with dermatitis or skin lesions, as it is considered to be purer and more hypoallergenic.
Envigo Diamond Dry and Shepherd Specialty Papers Alpha-dri are very similar cellulose products.
Envigo Sani-chip is a hard wood chip.
Shepherd Specialty Papers Cellu-nest is a twisted paper which the vendor obtains as a byproduct of coffee filter manufacture.
See **Appendix 20** for a complete list.

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

Bedding is received in sealed bags at the building loading dock. It is transported on pallets or carts on the freight elevator and stored in either the overflow room outside of the core, or each bag is wiped down with Clidox-S or Virkon-S before passage through the airlock. Once through the air lock the bedding is stored in a room off the clean cage wash area for easy access. Both storage areas are covered

by our vermin monitoring program. Sticky traps are placed to capture and identify insects and are visually inspected for fecal material around the room perimeter for any rodent presence. We have not seen evidence of feral/wild rodents in the core animal facility or SAF. The feed and bedding areas are temperature controlled and environment is monitored by a centralized alarm system monitored by OFM and security personnel.

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

Bedding is received either irradiated or is autoclaved prior to use. We purchase from vendors with a history of providing high quality products.

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.

Currently, the only transport of animals is from the SAF to PI labs and return, and when they are delivered to the PIs' laboratory or the core pathology suite at the end of the study. Animals remain in their home cage during transport and the cages are placed on carts that are sanitized in the animal facility. The cages are covered with a piece of drape material to minimize bright light and transport stress. The journey is between two connected buildings that does not require the animals be moved outdoors. Travel between floors is accomplished using a freight elevator.

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.).

Rats/Guinea pigs are provided shelters for enrichment. These are amber/red colored plastic tubes (circular or square) or a 'hut' type. Some rats are not permitted to have enrichment devices they can climb onto for reasons related to science. These

animals are provided a Nylabone as a chew toy. Lactating rat mothers with large litters are often provided a shelf for temporary escape.
Some mice are provided a shelf or hut. These devices are employed with mouse strains that 'play' with their water bottle to provide an escape, habitual food grinders, or breeding mice that appear to require more 'privacy'.
A HEPA vacuum is provided to clean up bedding and debris from Kleen benches, BSC's, fume hoods and caging change stations. Shop vacuums are available for routine clean up.
A complete list of equipment is provided in Appendix 22

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.

All rodents are housed on contact bedding in solid bottom cages. With the exception of one study where rats are housed in static micro-isolator cages, all rodents are housed on ventilated racks. This group of study rats are surgically implanted with a transponder and the static cages are placed on a specialized signal receiver platform that records cardiovascular data, and their cages are changed twice a week.
For animals housed in individually ventilated caging, group housed mice are changed at least every 2 weeks, but often weekly depending on cage density, and spot changed if wet spots are detected. Also, one study that treats single-housed mice with corticosterone must be changed more frequently (weekly) as the mice drink and urinate more frequently.

Group housed rats are changed twice a week on a Monday/Thursday or Tuesday/Friday schedule. Single-housed rats are changed 1-2 times per week based on size. Larger rats like the Sprague Dawley require more frequent cage changing to maintain the performance standard of clean and dry.
Guinea pigs are changed at least every 3rd day.

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

Periparturient mice, particularly some strains that are sensitive to disturbances which causes them to cannibalize their young, are left undisturbed for the first week after birth. This may result in the cage change interval being extended one time. However, if the cage is wet from a water bottle leak, it will be changed regardless of the timing of birth.

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

Soiled bedding within the animal facility with few exceptions is disposed of by transporting 'used' cages to the dirty side cage wash and dumping the cages in a ventilated bedding disposal station designed for this use which minimizes exposure to dust and allergens.
Once the cages are sanitized or in some cases autoclaved, the cages are bedded in one of the clean storage rooms.
One study transports mice that have been exposed to H3-thymidine on a drape covered cart to the PI's lab for final disposition. Her staff carefully bag up the soiled bedding inside a fume hood as described in the approved protocol and then coordinate disposal with the Safety Office.

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.

The only current exception is for rodent dams with new litters. To reduce stress, aggression and cannibalism, cages that are found with new litters are not changed for the first week, which could result in exceeding the 2 week maximum interval by a few days. Husbandry technicians are encouraged to pre-emptively change cages prior to expected birth dates to avoid this. However, cages that are observed to be wet are changed regardless, as chilling and mortality are of greater concern.

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).

Water temperature of mechanical washers is monitored by Temp-tape indicators placed with washer loads, by the tunnel washer's thermometer which shuts down the conveyor when temperature drops below 180F, and by chart recorders. Cages are visually inspected on the clean side and re-washed if visibly dirty. The filter tops, steel, bottles and sipper tubes are autoclaved after washing. The autoclave is monitored through the chart recorder, Temp-tapes, and periodic use of biological indicator ampules.

b) Describe preventive maintenance programs for mechanical washers.

Washers are serviced quarterly through a contract with Hydro-Kinetics who also provide on-call service. A representative of the vendor of chemical

supplies (Pharmacal) visits at least quarterly to calibrate chemical settings and monitor water hardness and pH.
The animal care staff performs the following on a routine basis:
Daily – Monitor and record temperature to ensure 180F
Weekly – for the tunnel washer, clean screens, flush, drain and refill tanks with fresh water. Every 4-6 weeks, clean screens, lubricate conveyors and clean the probes; for the rack washer, clean screens, lubricate, flush, drain and refill tanks, check spray arms, inspect and tighten the emergency stop cord and check the emergency exit.

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.

As described above, the majority of soiled bedding is dumped into a ventilated dump/disposal station located in the dirty side of the washroom (b)(7)(E). The bag is removed when full and at the end of the day. All refuse from the facility is disposed of at the end of the day in a commercial dumpster at the loading dock. Refuse includes soiled bedding and trash from receptacles throughout the animal facility.

ii. Animal carcasses.

Animal carcasses are disposed of as biomedical waste. The carcasses are stored in a freezer until the scheduled pick up day/time by a medical waste contractor (Stericycle).
Some experimentally naïve carcasses uncontaminated by drugs or chemicals (carbon dioxide euthanized only) are bagged, frozen and marked 'Raptor'. These are stored on a separate shelf and provided to a local raptor center for use as food for birds of prey.

g. [*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).

A newly contracted pest control program for the NIOSH buildings and premises began in August, 2019. The pest control operator (Tri-State) is certified and employs integrated pest management practices. He visits monthly and monitors for pest activity on the ground floor of the two main buildings and all around the premises. A Facilities Management employee checks and logs the two live traps on Ground floor daily. Vermin control in the animal facility (b)(7)(E) (b)(7)(E) areas includes visual inspection by animal care staff for evidence of feral rodents (feces) and insects and monitoring sticky traps to collect and identify insects. The contracted pest control operator can be called in to spray in core interstitial spaces if necessary. Chemicals are not used in the animal rooms or corridor. The ACTs place sticky traps in animal rooms and periodically collect and submit them to the AF Supervisor who prepares a report and sends to the AV for review. These are sticky boards versus a glue board, so there are not concerns about rodents getting caught on the traps. On rare occasions where mice escape from the handler, ACT's are trained to utilize the animal's thigmotactic behavior to capture it in an empty cage and if unsuccessful to then utilize a humane/live bait trap. Wild rodents have never been reported in the animal facility. The AF Supervisor manages the program and provides regular pest reports to the AV.

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.

Not applicable

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

To date, the only chemical applied (for controlling weevils, which sometimes appear in the summer) is applied outside of the animal facility and in maintenance core spaces. The product has been applied on an as needed basis by a contracted pest control operator and has been effective. If a more rigorous treatment were to become necessary and involve the animal rooms or facility corridors, a discussion would take place with users whose animals might be exposed, and a plan would be designed to minimize impact as much as possible.

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.

Animal care technicians rotate weekend and holiday duties. The Animal Facility Supervisor manages the schedule and provides 'notes' on medical cases and unusual events for the weekend technician. Typically, the AV will also review cases and concerns with the upcoming weekend tech near the end of the week. The ACT works full days on weekends and is granted compensatory time off the following Thursday and Friday. Holidays are handled similarly; the ACT gets another day off in the same pay period. While a rare event, if an ACT cannot come in on their assigned day, they swap duty with another technician who can work that day or the Supervisor will come in to cover. The Supervisor provides training to new hires by working alongside of them on their first assigned weekend. On their second weekend she is either available or working in office for ready access. The AV is available for troubleshooting if the Supervisor cannot be reached.
Daily observations are made, spot cage changes are performed as needed, feed and water is topped off as needed and additional duties are assigned by the Supervisor as time and census permits.

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

The animal care technician on weekend duty is a regular member of the AF staff. See previous sections on training and experience.

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

The phone numbers for the AV are posted in both air locks for easy access by both animal care and research staff. With rare exception, the AV is available by phone 24/7. If he cannot be reached, the backup veterinarian's contact information is posted as well. For planned absences, the AV notifies the IA and the Animal Facility Supervisor and provides an away message on email for NIOSH personnel. If the AV is out of town, the backup veterinarian is available for animal emergencies.

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

a. Identification

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

Cage cards are the primary tool to identify animals. Unique, sequential cage card numbers are generated through Topaz Elements. All offspring from breeding animals that require genotyping are tattooed to accurately identify the genotype for experiments and replacement breeding stock. PIs can request that animals be tattooed, ear tagged or ear punched if necessary to conduct their experiments. The procedure performed on experimental animals must be documented in an IACUC approved protocol. Ear punching/notching can be a simplified system to identify individual animals in group housing or more complex if each experimental animal requires a unique identifier. Animal tattoos are done with either an AIMS unit by the animal care technicians who are trained and certified or more recently using the Labstamp® unit. There are HELD SOPs for both of these methods.

b. Breeding, Genetics, and Nomenclature

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

Using our Topaz software system we can control what information goes into the database. The nomenclature derived from the vendors in the correct format can be entered into the system and allows the PI to choose from a drop-down list, which improves accuracy as this is the same system used to place animal orders. In addition to justifying the species, the PIs are frequently asked during protocol review to provide a rationale for choosing a specific strain, particularly when it comes to the use of genetically modified mice. Scientist members and the AV provide input on appropriate model selection in many cases and/or engage the PI on alternative choices.

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.

As above, the IACUC and AV have control over the Topaz database and can ensure that the correct nomenclature, vendor and stock number are entered to avoid keystroke errors by PIs. Strain divergence is discussed with PIs that utilize in-house breeding colonies during regular meetings between PIs, the AV and breeding specialist. Pedigrees are maintained on breeding colonies to determine generations, and phenotype abnormalities are tracked. Cryopreservation strategies have also been discussed and implemented in some cases. There is also an efficiency in tracking this information as all breeding colonies are maintained on a protocol held by the AV.

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

One of the animal care technicians has been designated as the breeding specialist. She has undergone special training and regularly views webinars provided on the subject. Pedigrees are maintained on all breeding colonies. Phenotypic abnormalities are tracked. Best practices for choosing replacement breeders to minimize bias are utilized for inbred strains. When replacement breeders have to be ordered from commercial vendors, the same source and background of the

original strain are procured to maintain continuity and avoid introducing mutations. SNP analysis has been discussed with users of long-term strains to determine if the strain has changed over time, but since many of these strains were not characterized from the beginning this tool may not be as helpful as it could be.

July 2020- Breeding colony strains are refreshed every 10-15 generations by back crossing to original parental or identical background animals procured from the vendor.

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."

The breeding specialist tracks phenotypes for all colonies. When new 'strains' are obtained, any background information that can be obtained from the source is reviewed at animal facility staff meetings, and staff are reminded to report any abnormalities until the strain can be characterized/defined under NIOSH housing environment. As above, all breeding is conducted under a protocol held by the AV, which provides more consistent care and oversight. If a colony of mice exhibit an abnormal phenotype that may be associated with pain/distress or a need for additional care, the breeding and research protocol may be amended to describe an updated plan to provide support and in some cases humane endpoints.

III. [*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 majority of rodents are obtained from commercial vendors who breed for research. Animals are evaluated by animal care staff upon entry for injury or illness that may prevent their use or jeopardize the health of resident populations. Health reports are reviewed from the vendor production areas, and certain areas are approved for future orders.
As quarantine space is limited and the risk of introducing rodent pathogens cannot always be predicted, the current recommendations to PIs wanting novel strains created or obtained from non-commercial sources is to send them for re-derivation through JAX or CRL. Cryopreservation can be conveniently done during the re-derivation process which ensures protections should breeding problems arise once the colony is established (contamination, failure to breed, spontaneous mutation, etc.)

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).

Animals arrive at the loading dock in climate-controlled vehicles from commercial vendors experienced in transport. The loading dock personnel contact the technician on call that day for deliveries. The shipping crates are passed from the vendor personnel to the ACT and examined for damage, which might indicate injury or contamination. The crates are loaded onto a cart and pulled inside the loading bay. The animal care technician checks to make sure the order is correct and complete based on the delivery schedule provided and signs off paperwork for the transporter. Any crates that are damaged are left in the bay, and the AV or AFS are contacted to determine disposition. Crates are transported from the loading dock on a cart up the freight elevator and to the AF airlock. The crates are wiped down with chemical disinfectant and passed through the airlock to a clean cart. The crates are placed in the animal room where the animals will be housed.

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.

Sentinel mice and rats are used to monitor for the presence of many rodent pathogens utilizing dirty bedding exposure on a quarterly schedule. Female mice and rats for this purpose are obtained from one of our vendors or transferred from one of our breeding colonies. The animals are pair housed and kept for approximately 6 months when they are replaced with a new naïve pair of mice. The animals are identified with a simple ear punch to ensure a different animal is tested at the 3 and 6 month time point. One pair of animals, socially housed, are assigned to each side of a ventilated rack, or in cases of low animal density to just one side of the rack. During cage change of the principal animals, a sample of soiled bedding with fecal material from each cage is added to a clean cage. Once all cages have been changed on that side of the rack, the soiled bedding in the collection cage is stirred and the volume either reduced if over-bedded or supplemented with clean bedding if under-bedded. The sentinel animals are moved to this dirty cage and must be housed for at least 24 hours. After 24 hours, if the ACT determines the cage is 'too dirty' or wet prior to the next scheduled cage change, the sentinels can be changed to a cage with clean bedding. At the 3 and 6 month time points, samples are collected for testing. A small blood sample is collected from the tail onto a testing card provided by the testing lab (IDEXX, Opti-Spot). Blood spot samples are tested for rodent pathogen antibodies. Feces for Helicobacter and other bacterial species and pinworms, and fur swabs for fur mites are tested by PCR. Feces may also be collected for testing from experimental or other breeding mice at the direction of the AV. See **Appendix 19** for agents tested.
Sentinels are not currently utilized for infectious disease studies and do not receive soiled bedding if study animal cages contain hazardous agents (e.g. during the 7 day period following tamoxifen dosing).
Biologics used by PIs are screened for rodent pathogens, typically with an IMPACT test. These include mouse and rat serum albumin used as part of the dispersion media

formula for pharyngeal aspiration dosing. Rodent antibodies that are injected into live animals are tested depending on their source and if the vendor can provide assurances through manufacturing process or testing that antibodies are free of rodent pathogens.

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

The animal facility has controlled access. Visitors are not allowed in if they have had contact with rodents in the last 72 hours. "Clean" pathogen free animals, feed and bedding are procured from reputable vendors to minimize the risk of pathogen entry. Animals that are transported out of the core AF may not be returned. An animal room remote from the core (SAF) is used for housing animals that must be taken to the PI lab for repeated experiments, and additional precautions are taken with regard to room entry order and PPE when staff provide daily service to this room. ACTs visit this room last and must shower back into the core AF or go home for the day. PIs with rare exception are not provided access to this room and core room animals at the same time. Health histories of animals from non-commercial sources are reviewed but are usually sent for commercial re-derivation. Direct procurement from such sources would also be dependent on the availability of quarantine space.
Supplies and equipment are sanitized before entry through the airlock, and high risk items that cannot be sanitized are not permitted.
Orientation of new research personnel includes the procedure for bringing items from the laboratories into the AF. The AF is equipped with dosing boards, heating pads, ear punches and bell jars as core equipment, which stay in the AF rather than being transported by multiple labs into the AF.
Filtered HVAC air, ventilated caging and use of cage change workbenches also reduce the risk of disease entry and transmission.
As described in the health surveillance section, biologics that may be rodent sourced or contaminated are tested, and specific lot numbers are subsequently approved for multiple lab use.

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

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

Damaged containers are not accepted, and any shipment of animals that does not conform to the order's specifications is reported and either refused or dealt with ad hoc before entrance into the AF. Shipping containers are examined and wiped down with disinfectant as they pass through the air lock. The crates are moved to the assigned room, and animals are examined as they are unpacked and transferred to the appropriate cages. Any abnormal or unhealthy animals are reported to the AV. The animals are provided fresh food and water and identified by cage cards placed on the cages. After all animals have been properly housed, the technician logs the information about the new animals in the room's logbook. Emptied shipping crates are sealed in plastic bags and removed from the AF for disposal. The preceding applies to all species (mouse, rat, guinea pig).

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.

All animals are purpose bred rodents. Newly arrived rats, mice, and guinea pigs are separated from other groups by housing them in individually ventilated cages. We seldom use quarantine procedures per se, because we rarely permit or accept animals that do not meet SPF standards for the entire facility. Recent health surveillance reports from vendors are evaluated, and rederivation is typically required when source colonies do not meet our standards. Animals of uncertain microbial status are tested repeatedly during quarantine before release to investigators. This is a rare situation.
Rooms external to the AF (b)(7)(E) can be used as quarantine or containment rooms, set up to allow housing of animals that cannot be brought into the core AF for reasons of infectious status.
Random source animals are not used.

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

In our Topaz protocol form we require that PIs provide a justification for use of an acclimation period less than 5 days.

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

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

All rodent species are housed in separate husbandry rooms with the exception of (b)(7)(E) Another, temporary exception is the recent housing of IL-4 KO mice, which had to be segregated and retested for a suspected pathogen, in the rat room (b)(7)(E) for a period of time in order to prevent exposure to other mice. They were housed in a separate IVC rack from the rats, and strict handling procedures followed, including opening and changing cages on different days. Rats and mice on protocols where repeated experimental interventions are required are housed in (b)(7)(E) until they reach the experimental endpoint. Animals are not permitted re-entry into the core facility. Mice and rat cage changes are handled on different days to address the few concerns in the literature on interspecies predation stress; however, some programs and commercial rodent vendors have been housing breeding colonies of rats and mice for many years without apparent effect. While the health status of our mice and rats is not exactly the same, they are both sourced as specific pathogen free and their health status is usually compatible.

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

Rats and mice are routinely housed together in one room outside of the core, (b)(7)(E) The animals are housed on different racks and changed on different days of the week.

c. Describe isolation procedures and related facilities for animals.

Two rooms could be used for isolation and are located outside of the core facility (b)(7)(E) However, rederivation is preferred for animals that do not meet our SPF criteria, and if an outbreak were to occur animals would likely be euthanized as the experimental results could not be relied upon.

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.

All animals are observed daily by animal care technicians that have been trained in husbandry and species-specific behaviors. Behavior issues related to health and disease for species and strains are routinely discussed during staff meetings as new projects and new models are approved. The change in quality of nest building is assessed for groups of mice as a measure of health. ACTs are required to report all abnormalities related to behavior, health and welfare using an electronic reporting system that generates a digital report that is sent to the AV, PI and AF Supervisor. The case is assigned a 'morbidity' number using two digits for the fiscal year and the next available number (e.g. 20-0139). A sticker with this 'case' number is placed on the cage card of the affected animal. Green stickers are for active cases, yellow for cases under observation only and red is for cases that are cleared. If a case is reactivated, a green sticker replaces the red. When the case is cleared, the sticker remains and the record can be re-initiated to maintain case continuity. In addition, ACTs are instructed to contact the veterinarian directly if animals require attention and/or treatment, especially on weekends and holidays when they are working alone. ACTs are permitted to treat animals under veterinary order or per an AV-approved treatment plan (e.g. dermatitis). Cases are cleared by the AV or after discussion with the AV. In an emergency the backup veterinarian can be contacted if the AV is unavailable.

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

ACTs are required to report all abnormalities related to behavior, health and welfare using an electronic reporting system that generates a digital report that is sent to the AV, PI and AF Supervisor. In addition, ACTs are instructed to contact the veterinarian directly if animals require attention and/or treatment. Cases are discussed during regular staff meetings. However, the AV is available outside of regular meeting times on a daily basis to discuss and resolve clinical cases.
Research staff are encouraged to contact the AV directly if they have concerns regarding the health and wellbeing of their research animals, including obtaining unexpected results. The animal care staff have a good working relationship with the research staff and will frequently discuss findings with staff while in the animal facility. During orientation, research staff are informed that they are required to make notations in the room logbook when they enter a room. The ACTs review the previous day's notes and bring concerns to the attention of the AV or AF Supervisor.

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.

As a rodent only facility, the preventive medicine program is structured around the acquisition of healthy, pathogen free animals from reputable vendors. Sentinel animals are used to detect the incursion of pathogens that are spread by the fecal-oral route. Environmental testing for pathogens is under consideration, however recent information presented at national AALAS seminars doesn't indicate it is any more reliable than PCR testing of pooled feces.

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.

The AV is available 24/7 and is less than 4 miles from the facility. The AV has a cell phone and is available by phone, text and email. The CDC intranet Skype IM function has also been helpful for communication in text, and IM can be reviewed more easily during meetings. If for some reason, the AV cannot be reached, the primary backup

veterinarian, who is also a full time scientist, can be called and her phone numbers are located in the AF.
During planned absences, arrangements are made with one of the backup veterinarians to cover, and an email is sent to the animal facility staff, the IACUC Chair and Administrator, in addition to an out of office message on the email account informing these groups of who is providing emergency service.

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

Consistent with the Guide and AWRs and stated in the NIOSH PHS Assurance, "The Division Director delegates to the AFD/AV responsibility for overall management of the animal care program with operational oversight of animal care staff and IACUC support staff. He establishes and maintains adequate programs of veterinary care and reports directly to the IO on matters of animal care and use. He serves as Attending Veterinarian and as such is an *ex officio* voting member of the CDC-Morgantown (NIOSH) IACUC." As such, the AV has the authority to provide treatment (including euthanasia) to animals. The procedure is to make a reasonable attempt to contact and discuss the case with the PI, but the AV has the authority to euthanize for humane reasons and to prevent suffering.

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.

An electronic records program which was designed in house is used to initiate and track cases. The case is assigned a unique identifier with the fiscal year and the next available number. This number is indicated on the cage card, so if the case is cleared and then needs to be reactivated, the animal or group of animals receive continuity of

care and have a medical history. Entries can be made by the ACT and AV to document care and facilitate communication. Once the case is cleared it is archived but can be retrieved if the animal is reported for a health issue in the future. If treatment is required, 'treatment cards" are generated by the supervisor and the ACT records/ documents that the treatments were performed as scheduled.

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.

The AF Supervisor is responsible for maintaining the database. Outstanding records are identified and updated during staff meetings. The AFS works with the IT contractors to make changes, add and delete animal care personnel and troubleshoot the program. Only the animal care staff have access and the ability to edit records.

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

The AV receives electronic notification of cases and updates. He can make notations for treatment or follow-up in the record, and clear and archive cases.

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

a. In-house diagnostic laboratory capabilities.

NIOSH has a highly skilled Core Pathology group available for necropsy and histopathology. For animals that are found dead outside of normal working hours or when staff in necropsy is not available, ACTs are trained to perform basic gross necropsy and place samples in formalin for follow up (b)(7)(E). We have access to limited clinical pathology support from the (b)(7)(E) Molecular Biology Core, which has hematology machines that can run blood chemistries and cell counts. There are no other in-house diagnostic laboratories available.

b. Commercially provided diagnostic laboratory services.

(b)(4) is the primary laboratory used for processing sentinel animal samples. (b)(4) has been used previously. These vendors provide antibody serology testing, PCR, microbiology and necropsy services if needed.

c. Necropsy facilities and histopathology capabilities.

Two veterinary pathologists are available for consultation when necropsy is needed for clinical cases. There are two skilled prosectors in the Core Pathology lab (b)(7)(E) to perform necropsies and prepare histopathology slides. The AV will necropsy animals and collect tissues when they are not available, especially on weekends and holidays.

d. Radiology and other imaging capabilities.

Not available.

5. Drug Storage and Control

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

Over-the-counter and prescription drugs are ordered and inventoried by the AFS under the AV's veterinary license. They are stored in a locked cabinet in the AFS's office (b)(7)(E). Aliquots are dispensed to ACTs as needed to be kept in the animal room until treatment is discontinued.

b. Describe record keeping procedures for controlled substances.

Controlled substances for the Animal Facility are now ordered by the AF Operations Officer under the AV's DEA license and inventoried by the AFS and stored in a safe that was recently installed in her office (b)(7)(E) Controlled substances for HELD researchers are procured under a DEA registration held by a division scientist who is responsible for the distribution and records outside of the animal facility. Primarily euthanasia solution is dispensed to research staff who are responsible for recording use

and returning logs and excess product back to the biologist who oversees the program for the registration holder. All controlled substances in HELD are now inventoried on a secure website (CISPro Global), which is managed by the HELD Quality Assurance Unit (QAU). The new HELD SOP-OD-00346 stipulates the proper ordering, handling and use of Schedule I and II controlled substances. The QAU also manages the biennial consolidation of expired controlled drugs in HELD for reverse distribution.

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.

Pre-surgical planning begins with the development of the animal protocol, when procedures are described, plans are made for anesthesia and peri-surgical care, and personnel are identified. Laboratory staff planning for surgery are responsible for equipment and supplies but may consult the AV and arrange for use of some core equipment and facilities. The AV is consulted during development of the protocol and reviews all protocols. The IACUC is responsible for ensuring that personnel are adequately trained in surgery, care, and anesthesia. The surgical program is overseen by the AV, and the animal care staff monitors postsurgical animals. The investigator's surgical team is responsible for planning to monitor and care for the animals during surgery, anesthesia, and peri-surgical periods.

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.

Only rodents are used in this program. There have been no experimental survival surgeries performed in HELD for more than a year now. The AV occasionally conducts clinically necessary minor survival procedures or sedates animals for detailed examination in (b)(7)(E)

Room (b)(7)(E) in the AF is equipped and operated for rodent surgery and other aseptic procedures. Equipment includes isoflurane gas anesthesia, oxygen, PhysioSuite physiological monitor, a heat lamp and heated operating platforms. Requirements for aseptic surgery are sterilized scrubs and gown/lab coat, head cover, 2nd pair of shoe covers and gloves. The room is sanitized after use and is on a regular housekeeping schedule. (b)(7)(E) is mainly used for experimental dosing (e.g. oropharyngeal), and for tattooing and collecting tissue samples for genotyping.
Procedures performed in (b)(7)(E) are:

1. Rat – tail and ear biopsy and blood collection for genotyping
2. Mouse – experimental dosing, tail and ear biopsy for genotyping, tail tattooing

Non-survival surgery is performed in these research laboratories with dedicated lab bench areas to perform these procedures:

1. Rat - placement of intravascular catheters, laboratory (b)(7)(E) This lab is equipped with an isoflurane anesthesia machine and oxygen with a downdraft work surface to minimize anesthetic gas exposure of the operator. ECGs and blood pressure are monitored as part of the experiment and also aid the operator in establishing adequate levels of anesthesia.
2. Rat - tracheotomy for invasive plethysmography (Buxco), laboratory (b)(7)(E)
3. Rat – tracheotomy for pulmonary function (Flexivent) (b)(7)(E)

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).

Major surgery is defined as a surgery that enters a major body cavity such as the abdomen, thorax or brain or causes permanent impairment or disability such as loss of limb or limb function. Laparoscopic procedures have not been performed at NIOSH so there has been no debate on if this approach would be major or minor. Minor surgery is limited to tail biopsy for genotyping which has been replaced for most breeding colonies by ear punch tissue, ear snips, or blood collection in the case of the CMT rats.

b. How is non-survival surgery defined?

Non-survival surgery has been defined as surgery from which the animal does not recover consciousness prior to euthanasia while under anesthesia.
The IACUC has determined that terminal blood collection (percutaneous or invasive) and whole body perfusion following a fatal dose of euthanasia solution (after it has been established that the animal has been rendered unconscious based on observation of slowed or lack of breathing activity and lack of withdraw reflex from firm pressure of the paw) is a terminal procedure as part of euthanasia and not surgery. The perfusion/exsanguination are performed as a single procedure in conjunction with euthanasia which is consistent with guidance provided during the OLAW webinar on non-pharmaceutical grade chemicals in March 2012 and is consistent with our observations that the animal is near death and unconscious at the time of the event.

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

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

For rodent only survival surgery.

Following anesthetic induction and stabilization on maintenance anesthesia, the patient is prepared for surgery.
Patient preparation includes removal of hair with #40 or 50 clipper blade or razor from surgical site, cleaning of the surgical area with disinfectant (betadine) and alcohol at least twice in a pattern to avoid recontamination of the surgical site taking care to minimize wetting and chilling the patient. The surgical site is draped with a sterile drape. Instruments are autoclaved and laid out on a sterile drape. A new set of sterilized instruments are used for each patient.
Transponders/implants are sterilized by autoclaving if possible or by use of chemical sterilant as described by the vendor. Devices are rinsed in sterile saline prior to placement.
The surgeon is expected to wear sterilized scrubs and PPE, thoroughly scrub/wash hands, dry them with a sterile towel, and don sterile surgical gloves.

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.

With proper pre-surgical planning, sufficient numbers of surgical packs are wrapped, sterilized and staged for the procedure(s). Ideally, a separate set of instruments are autoclaved in a pack for each patient. Glass bead dry sterilization of thoroughly cleaned instruments is permitted for minor procedures of a repetitious nature. The unit must be operated per manufacturer's instructions. For instance, the Germinator 500 must be heated up to 233C and instruments left in the hot beads for at least 15 seconds. The surgeon is dressed in sterilized scrubs and shoe covers upon entry to the surgery room. They must don a sterilized 'lab pack' containing a lab coat, bouffant, surgeons mask and second pair of shoe covers. Following patient preparation and hand washing, sterile surgeon's gloves are donned.

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

The telemetry devices are recovered from cadavers and re-used. The device is cleaned and chemically sterilized per the manufacturer's instructions using Cidex/ glutaraldehyde followed by rinsing in sterile saline.

d. Indicate how effectiveness of sterilization is monitored.

Temperature sensitive indicators are used inside each pack. Autoclaves are monitored by the Safety Office using a biological indicator test.
No infections have been noted at surgical sites at necropsy when devices are recovered.

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

There is a dedicated room (b)(7)(E) in the animal facility that is used for survival rodent surgery. The room is limited access and is cleaned and sanitized by AF staff on a regular basis. Medical oxygen, isoflurane vaporizer, Physiosuite monitor, heated surgery platforms and recirculating water heating pads are available for use. Veterinary consultation on surgery, anesthesia, analgesia and post-operative care planning is available to all scientist during protocol development and ongoing as part of PAM.

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.

Rat survival surgeries: Surgical plane of anesthesia is determined by lack of pedal reflex (absence of limb withdrawal with firm pinch to toes/paw). Heart rate and respiratory rate/depth are observed and recorded using Physiosuite monitor by Kent Scientific. Body temperature is monitored by a rectal probe and maintained by a heated surgical platform. Written records are kept noting depth of anesthesia, respiratory or other physiological parameters, drugs or fluids administered, and experimental data. Records of experimental data are usually made electronically as well. There are currently no survival surgeries being performed on active protocols.
Rat non-survival procedures: The lack of pedal reflex is used prior to any incision. Other parameters often are monitored as part of pulmonary and cardiovascular studies.

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.

The investigator's surgical team is responsible for monitoring and caring for the animal during the peri- and postsurgical recovery, and for keeping appropriate records as detailed in the IACUC-approved protocol.

The animal is kept warm and observed until fully upright and ambulatory. Analgesics are given during surgery and during the postoperative period. The animal is observed for body functions (eating, drinking, and elimination), signs of incisional and generalized pain, distress, or excessive attention to the surgical wound; and the wound is observed for integrity and inflammation.

Head cap rats are received from the vendor approximately 1 week post-operative but daily care and monitoring of the head cap incision site is required for the life of the animal. The animal care staff provide this care. This study was completed in 2018; there are currently no head cap rats on active protocols.

Documentation of monitoring and analgesia is made in the room log book. Complications are reported to the AV by the research staff or animal care staff.

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

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

For surgical cases, the research staff is responsible for assessing pain and distress, however the animal care staff also observe these cases daily and report pain/distress if observed. Pain is assessed at cage side by observing the animal's general demeanor (attitude, activity, appetite, water intake and eliminations). While rat pain scales are not used specifically for the current surgery projects, signs of writhing, guarding and piloerection have been documented to be associated with abdominal pain. The incision site is observed for redness, swelling or drainage and is palpated to determine if pain is present. Animals for which pain cannot be controlled are euthanized.

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

Some animal care staff and research staff are trained through previous experience. Cases that are brought to the attention of the AV are frequently evaluated by both parties to compare findings and make assessments. This practice ensures that staff are making comprehensive evaluations and making correct assessments for pain. Interpretation of clinical signs associated with pain and distress specific to species is discussed during bimonthly meetings between the AV and animal care staff. Specific rat behavior is noted above. Staff are also assigned specific modules in the A.L.L. covering specific species, rodent pain and post-procedural care.

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.

Isoflurane by precision vaporizer – rats and mice
Isoflurane by open drop - mice
Brevital –methohexital - rats
Ketamine/xylazine cocktail – rats and mice
Ketamine/xylazine/acepromazine cocktail - mice

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

The AV reviews all IACUC protocols and amendments. He is available for consultation regarding choices of anesthesia, analgesia and palliative care.

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.

Rats instrumented with telemetry transponders are surgically altered by an AAALAC accredited vendor (Hilltop Laboratory Animals) and now arrive at least ten days post-operatively. Continued analgesia has only been required a couple of times. This issue has since been addressed with the vendor, and the modified contract stipulates a longer recovery time to ensure healing and full recovery before shipping.

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.

Neuromuscular block agents are no longer used.

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

All anesthetic vaporizers are serviced annually by an outside contractor. Arrangements are made by the Animal Facility Supervisor. Vaporizers are labeled with the service date.

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

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

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

The IACUC has a policy on euthanasia and relies on methods described in the AVMA Guidelines on the Euthanasia of Animals: 2013. The primary method of euthanasia for most animal studies is barbiturate overdose administered by the intraperitoneal route. CO2 overdose is the primary method for AF colony animals. Other methods include exsanguination under anesthesia, conscious decapitation, and microwave radiation. A secondary method to ensure death is required for barbiturate and carbon dioxide overdose. Neonatal mice and rats must be dosed for an extended period of time when carbon dioxide is used. Scissors can be used for decapitation of neonatal mice and rats less than 7 day of

age, but a guillotine must be used otherwise. While not counted as animals against a protocol, rodent fetus work must describe how feti will be processed since term pups may experience pain if allowed to become conscious.

July 2020- The 2020 AVMA Guidelines on the Euthanasia of Animals have been fully instituted and our Euthanasia Policy (P-0003) updated. CO2 euthanasia stations have all been upgraded with a second, factory calibrated flowmeter to ensure a flow rate of 30-70% volume displacement per minute, and posted instructions have been updated.

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

The IACUC has a euthanasia policy which covers the methods used at NIOSH. Stations used for carbon dioxide euthanasia are limited and must be approved by the AV including pilot testing the unit to ensure it is operating correctly. Systems have been equipped with pressure regulators and flowmeters to reduce flow rate as indicated in the 2013 Guidelines for Euthanasia. Appropriate flow rates and instructions are posted with the apparatus. There is a HELD SOP for the AIMS shoebox euthanasia stations and a recent AF Working Standard for the Euthanex SmartBox system in (b)(7)(E)

Guillotines: The IACUC requires a log of use, and it is resharpened after 250 uses or once per year.

The microwave apparatus is maintained by the one investigator whose laboratory uses it.

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

A secondary method to ensure death is required when barbiturate overdose and carbon dioxide are used. The secondary methods currently approved by the IACUC and consistent with the most recent version of the AVMA Guidelines for the Euthanasia of Animals are described in the animal care and use protocol. These methods include immediate harvest of organs (necropsy), complete exsanguination by transection of major vessels,

decapitation, cervical dislocation, and bilateral thoracotomy. These secondary methods require the operator first determine that the animal is unconscious by lack of pedal reflex.

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 Animal Facility core and associated Inhalation Facility are located on the (b)(7)(E) which is connected by an internal bridge to (b)(7)(E) where most of the HELD laboratories are located (b)(7)(E) The AF core is equipped with a dedicated air handler with humidifying capability. There is one satellite room managed by animal facility staff on the (b)(7)(E) The AF core and Inhalation facility contain 6 rooms used to house rodent breeding colonies; 9 rooms used to house rodents on study (some of which have chemical fume hoods) for animal dosing; 1 animal housing room is reserved for BSL2 animal work with a Class II biosafety cabinet; 3 rooms with multiple cubicles for inhalation exposures; 8 additional procedure rooms that are used for aseptic rodent procedures, necropsy, non-invasive plethysmography, robotic welder and more. There are 4 storage rooms for equipment including 2 for clean caging. One of these rooms can also be repurposed for animal housing or quarantine room if necessary. Three rooms are used for feed and bedding storage, one within the core and two outside of the core. Within the core are a clean and dirty side cage wash area. Additional support areas include an animal care staff breakroom, restrooms/locker rooms and some office space and computer space. A wireless internet system was installed building wide in December 2015.

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

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

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

1. General arrangement of the animal facilities (conventional, clean/dirty corridor, etc.).
2. Physical relationship of the animal facilities to the research laboratories where animals may be used.
3. Types of available animal housing spaces used, such as conventional, barrier, isolation/quarantine, hazard containment (infectious, radioactive, chemical), "animal cubicles" or facilities specifically designed for housing certain species such as ponds, pastures, feedlots, etc.
4. Finishes used throughout the animal facility for floors, walls, ceilings, doors, alleyways, gates, etc. (note any areas that are not easily sanitized and describe how these are maintained).
5. Engineering features (design, layout, special HVAC systems, noting exhaust air treatment, if applicable) used in hazardous agent containment.
6. Security features, such as control of entry, perimeter fences, gates, entryways, cameras, guards; identify and describe exceptions for individual facilities or areas incorporating fewer or additional security features than the general features described.
7. Consideration for facilities with exterior windows, if applicable, including management of environmental conditions (i.e., temperature and photoperiod control) and potential security risks.
8. Storage areas for flammable or hazardous agents and materials (e.g., disinfectants, cage-washing chemicals, pesticides, fuel).

1. The AF core is arranged in a U-shaped configuration with a single corridor and a general layout of clean to dirty (i.e. the clean side of the wash room is at the top of one side of the 'U' and moves to the other side of the 'U' to the dirty side of the washroom. Clean equipment from the clean side of the wash is stored near the clean washroom. Supplies are brought in through the clean side air lock. Soiled cages and racks enter through the airlock on the dirty end. Almost all rodents are housed in IVC caging. The

Inhalation facility (IF) 'T's' off of the AF core near the air lock at the dirty end of the 'U'. There are two animal husbandry rooms and several procedure rooms related to inhalation exposure research. The IF procedure rooms are equipped with 3 ventilated cubicles for exposure.

Outside of the AF core across the main hallway that crosses (b)(7)(E) are three rooms used for storage (feed, bedding and equipment).

On the ground floor of the same building is the shared loading dock with easy access to a freight elevator.

2. The AF core is located on the (b)(7)(E) and most of the research laboratories are located on the (b)(7)(E) The two buildings are connected by internal bridges. There are shared freight elevators in both buildings for transport of animals and animal husbandry related equipment movement.
3. With the exception of rodent breeding rooms, most of the research exposures utilize potentially hazardous agents. A single room has been set aside for BSL-2/ABSL-2 biological exposures (b)(7)(E) The animal facility is access controlled and individual rooms are equipped with badge readers to control access to individual animal/ procedure rooms in the AF core. Most of the rooms are block walled or sheet rocked walls, some with cabinetry. Animals are housed on ventilated racks exhausted directly to the building exhaust or HEPA to the room (Tecniplast® units). Animal rooms in the IF and the one satellite room (b)(7)(E) are designed the same way. Cubicles are used in the IF procedure rooms but for exposures only, not housing.
4. Floors in rooms and corridors are epoxy resin type installed over elevated, cast in place concrete slab. All walls are skim coat plaster on CMU block or cement board with biolastic polymer paint. Ceilings are sealed, water resistant drywall with biolastic polymer paint. Aluminum-alloy wall rails line all of the corridors. All room doors also have railing in the face and a 4 ft. corner roller on each frame to protect the finishes. Corridor corners are protected from floor to 8 ft. with 4"x4" stainless steel corner guards. Stainless steel fire alarm guards were recently installed on walls. Walls in the cage wash area have been reinforced with sheets of stainless steel. The animal room doors are 3 ft. wide with an additional 1 ft. dead leaf, are 7 ft. high, of hollow steel construction with a view panel. The view panels on most doors contain enclosed blinds in order to exclude extraneous light. All exterior windows are in the hallway and are of double pane construction. A renovation project in September 2016 replaced

most of the glass windows on the west facing side with insulated panels to reduce outdoor light and improve temperature regulation in the corridor.

5. The AF and IF are supplied by a dedicated air handler. The system utilizes steam and chilled water with humidity control. The satellite room, (b)(7)(E) is on a different air handler with no humidity control. Rooms are individually regulated. Environmental control, both settings and monitoring, are managed by OFM through an automated system. Relative pressure can be set to positive or negative depending on the room function (clean vs hazard). Ventilated animal racks supply and exhaust air is HEPA filtered and most racks are exhausted directly to the building exhaust. Additionally, hard ducted chemical fume hoods are available in some animal rooms for administration of hazards. Cubicles where pulmonary exposures are performed in the Inhalation area are negative to the procedure room, and the procedure rooms are negative to the corridor.
6. The entire NIOSH facility is access limited and all employees are required to wear their photo ID badge. Regular employees may gain access through limited entry points that are monitored by camera. After hours and weekends all employees must enter through the security station and sign in and out. A security staff is onsite 24/7/365. Security staff routinely patrol through the building including the animal AF and IF corridors after hours. The AF is located on the (b)(7)(E) making direct entry from the outside unlikely. Cameras are strategically located throughout and around the building and monitored by the security staff. The loading dock area is surrounded by a perimeter fence and is locked outside of regular work hours and during security drills. Fire evacuation drills and shelter in place drills for emergencies or active shooters are held throughout the year.
7. The east and west walls of the AF core have windows. A renovation project in September 2016 replaced much of the glass on the west side across from animal rooms with insulated panels to reduce light incursion to animal rooms and to better control temperatures in the corridors. The windows are an unlikely entry point as the AF is on the 3rd floor. Light level incursion into the animal rooms is minimized by the use of blinds and is measured twice a year (reports are available during visit).
8. Storage for flammable agents in the AF core is limited to the flammables' cabinets in (b)(7)(E) (cage wash).

July 2020- Tecniplast IVC units are directly exhausted into ceiling ducts, using a simple air gap insert that has proven to be quite suitable; whereas, some of the Allentown units in our breeding rooms are still exhausted through HEPA into the room.

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.

Our criteria for a satellite facility follows the PHS Policy of a containment outside the core facility in which animals are housed for more than 24 hours. This is detailed in IACUC Policy-0005, requirements for a Satellite Animal Facility (SAF) and holding animals in laboratories for <24 hours. Only one room, (b)(7)(E) is approved for satellite housing of rodents. It is approved for >24 hour indefinite housing of both rats and mice. The need for a handful of investigators to perform repeated interventions on experimental animals and return to housing is the rationale for animals to be housed outside of the AF core. This room can also be re-missioned to function as a quarantine facility outside the AF core.

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.

The IACUC authorizes the SAF in Policy-0005, which is periodically reviewed. Compliance with Guide standards is required, with the understanding that (b)(7)(E) is often incapable of maintaining >30% RH. The IACUC inspects this room and the laboratory around it as part of the semi-annual inspection process and reviews records. The SAF is centrally managed and all AF staff have badge access to the room; it gets the same routine husbandry care as AF core animal rooms. For security, the SAF is nested within a larger laboratory (b)(7)(E) also with badge access; lab areas are patrolled by security staff 24/7.

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.

NIOSH is equipped with a diesel-fueled emergency generator that is regularly checked and maintained by OFM staff Emergency power is supplied in all animal rooms to ventilated cage racks or other critical equipment. In the event of a power failure, ventilation and heating capability are maintained, and outlets supplying ventilated racks etc. receive emergency power after a 10 second delay. The institution is normally supplied with power from a substation located within 1000 ft. of the facility. This substation has multiple feeder options in the event of a failure, and the feed from this source is buried and encased in concrete.

Power interruptions are rare and usually have been planned internal shut-downs related to maintenance or renovation, with provision made for power and HVAC to maintain adequate conditions in animal areas. Power interruptions are recorded in room logbooks, and investigators are notified.

July 2020- We endured an overnight power outage, including to backup/emergency power, in March of this year. Power was out for nearly 24 hours, and all animal housing rooms were dark and quiet. The AV and staff checked on animals the evening of and the following morning, and checked them closer when power was restored. The rooms and cages got pretty stuffy, but remarkably logged temperatures were not higher than 77F, RH remained in range, and all animals appeared to be fine and unaffected afterward.

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).

Not applicable.

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.

One PI maintains a microwave euthanasia unit (b)(7)(E) that is used and maintained by the PI's research staff. While there are no current collaborations, past and future collaborations have allowed for microwave euthanasia of mice and rats from (b)(7)(E)

2. **Other Animal Program Support Facilities**
Describe other facilities providing animal care and use support, such as feedmills, diagnostic laboratories, abattoirs, etc.

A core necropsy/histopathology laboratory (Core Pathology, room (b)(7)(E) is located in the lab wing. There are multiple downdraft necropsy tables, chemical fume hoods and an adjacent room for tissue processing. The core is overseen by a veterinary pathologist and staffed with highly skilled laboratorians to perform end of study processing.
The Molecular Biology Core facility (b)(7)(E) provides limited clinical pathology support.

Appendices (1-22) are provided in a separate file.

NIOSH - 000692
Appendix Table of Contents

Appendix 1: Glossary

AALAS American Association for Laboratory Animal Science

AAS Associate of Applied Science

ABSL Animal Biosafety Level

ACIB Allergy and Clinical Immunology Branch

ACLAM American College of Laboratory Animal Medicine

ACT Animal Care Technician

ACU Animal Care and Use

ACUC Animal Care and Use Committee

ACUPO Animal Care and Use Program Office

AED Automated External Defibrillator

AF Animal Facility

AFA Animal Facility Assistant

AFD Animal Facility Director

AFS Animal Facility Supervisor- Contractor

ALAT Assistant Laboratory Animal Technician

ALL AALAS Learning Library

AV Attending Veterinarian

AVMA American Veterinary Medical Association

AWRs Animal Welfare Regulations

BEB Biostatistics and Epidemiology Branch

BMBL Biosafety in Microbiological and Biomedical Laboratories, 5th edition

BSL Biosafety Level

CDC Center for Disease Control and Prevention

CMAR Certified Manager of Animal Resources

CPSC US Consumer Product Safety Commission

CVM College of Veterinary Medicine

DACVPM Diplomate American College of Veterinary Preventive Medicine

DEA Drug Enforcement Agency

DMR Designated Member Review or Reviewer

DVM Doctor of Veterinary Medicine

EAB Exposure Assessment Branch

ECTB Engineering and Control Technology Branch

FCR Full Committee Review

FDA Food and Drug Administration

GSA General Services Administration

HELD Health Effects Laboratory Division

HEPA High efficiency particulate air

HOBO® data logger and software from Onset Computer Corporation, is not an acronym.

IA IACUC Administrator

IACUC Institutional Animal Care and Use Committee

ICCVAM Interagency Coordinating Committee on the Validation of Alternative Methods

IF Inhalation Facility

IHT Inhalation Facility Technician

ILAM Institute for Laboratory Animal Management

IM Instant Messaging

IO Institutional Official

Appendix 1: Glossary of Abbreviations and Acronyms

IVC Individually Ventilated Cages

JOVE Journal of Visualized Experiments

LATG Laboratory Animal Technologist certification

LD50 Lethal dose that kills 50% of a test sample

MOU Memorandum of Understanding

MPH Master of Public Health

MUB Morgantown Utility Board

N/R/P N= not resistant to oil; R= somewhat resistant to oil; P strongly resistant to oil (OSHA classification for respirators)

NC3Rs National Centre for the Replacement Refinement and Reduction of Animals in Research

NEFF New Employee, Fellows and Facility Users (form)

NIEHS National Institute for Environmental Health Science

NIH National Institutes of Health

NIOSH National Institute for Occupational Safety and Health

NTP National Toxicology Program

OADS Office of Associate Director for Science

OAMS Office of Administration and Management Services

OD Office of the Director

OFM Office of Facilities Management

OHP Occupational Health Program

OLAW Office of Laboratory Animal Welfare

OSHE Office of Safety, Health and Environment

PAM Post approval monitoring

PHS Public Health Service

PI(s) Principal Investigator(s)

PPE Personal protective equipment

PPRB Pathology and Physiology Research Branch

QAC Quaternary ammonia compounds

RLATG Registered Laboratory Animal Technologist

RVT Registered Veterinary Technician

SDS Safety Data Sheets (formerly MSDS)

SLC solute carrier gene family

SNP Single nucleotide polymorphism (analysis)

SOP Standard Operating Procedure

SPF Specific pathogen free

TMBB Toxicology and Molecular Biology Branch

USDA United States Department of Agriculture

USP United States Pharmacopeia

VMD *Veterinariae Medicinae Doctoris*

WVU West Virginia University

Appendix 2.1: Summary of Animal Housing and Support Sites

Animal Facility Space Key		
Building	Room #	Category	Function
(b)(7)(E)		Animal Housing	Breeding, Mouse
		Animal Housing	Breeding, Mouse
		Animal Housing	Breeding, Mouse
		Animal Housing	Study room, Barrier/ABSL2
		Animal Housing	Breeding, Mouse
		Animal Housing	Breeding, Rat
		Office	AF Technician admin
		Animal Housing	Study Room, Mixed
		Animal Housing	Study Room, Mouse
		Animal Housing	Study Room, Mouse
		Storage	Clean caging storage
		Animal Housing	Study Room, Mouse
		Animal Housing	Study Room, Mixed
		Animal Housing	Study Room, Rat
		Animal Housing	Study Room, Telemetry rats
		Animal Housing	Study Room, Mouse
		Office	AF Supervisor
		Office	vacant
		Office	AF Operations Assistant/Officer
		Office	AV
		Office	IA
		Office	Inhalation Tech Staff
		Procedure	Sterile surgery & procedures
		Procedure	Study Room, Rat
		Procedure	Necropsy, CO2, Freezer, Refrigerator
		Procedure	Chambers
		Exposure	Inhalation Chambers
		Exposure	Inhalation Chambers
		Exposure	Robotic welder
		Procedure	Inhalation Lab
		Procedure	Inhalation Chambers
		Exposure	Thermal (metallic) Spray Coating
		Procedure	Non-Invasive plethysmography
		Storage	Feed/Bedding
		Storage	Clean cage storage
		Storage	Breeding, mouse
		Storage	Equipment, food & bedding
		Storage	Feed/Bedding
		Storage	Specialized feed in shared cold room
		Storage	Vestibule, flammables storage
		Support	Clean cage wash, sterile storage, bottle
		Support	Dirty cage wash area

Appendix 2.1: Summary of Animal Housing and Support Sites

(b)(7)(E)	Support	Breakroom
	Support	Restroom
	Support	Locker Room
	Support	Restroom
	Support	Clean cage
	Support	Locker Room
	Support	Corridors
	Laboratory	Histopathology Core
	Storage	Caging equipment, supplies
	Storage	Carcass freezer in shared lab

Appendix 2.2: Summary of Animal Housing and Support Sites

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

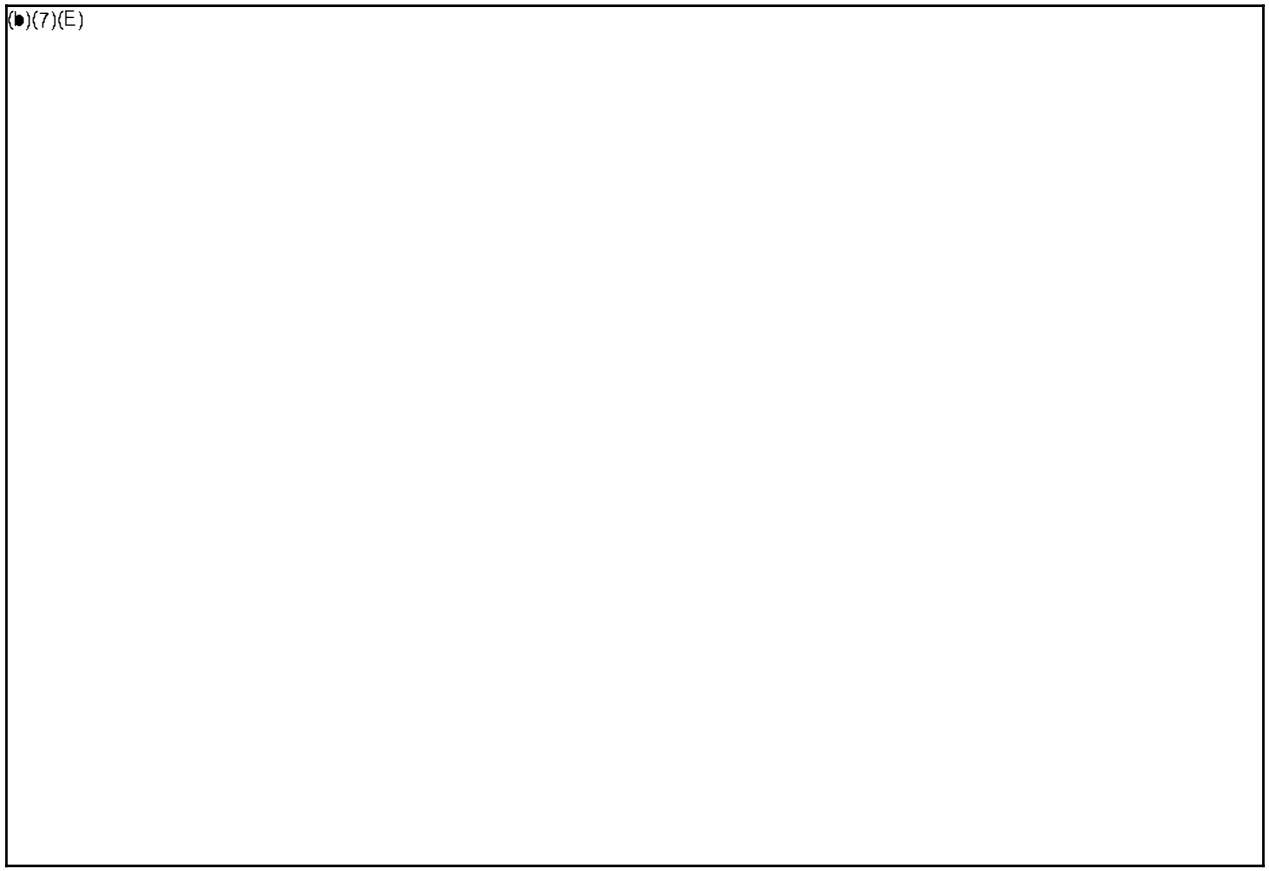
Animal Housing and Support Sites						
Location (building, site, farm name, etc.[a])	**Distance from main facility[b]**	**Approx. ft², m², or acreage for animal housing**	**Approx. ft², m², or acreage for support or procedures**	**Species housed**	**Approx. Daily Animal Census by species**	**Person in charge of site**
(b)(7)(E)		3080	7000	Mice Rats Guinea Pigs	1950 290 0	(b)(6)
	See line drawings	240	0	Mice Rats	20 16	
	See line drawings		152	Storage	0	
	See line drawings		126	Storage	0	
	See line drawings		194	Storage	0	
	See line drawings		123	Storage	0	ACIB (b)(6)
	3 miles		800	Storage	0	OAMS

Totals:	**3320**	**8395**
Total animal housing and support space:	**11715 Ft²**	
	(please specify ft² or m²)	

[a]Please state name and/or use acronyms described in **Appendix 1** for building names, if not coded for confidentiality.
[b]Campus or site map(s) may also be provided in lieu of this information.

Appendix 3.1: Line Drawings
Animal and Inhalation Facility Space

Request that all line drawings showing identified room locations be redacted

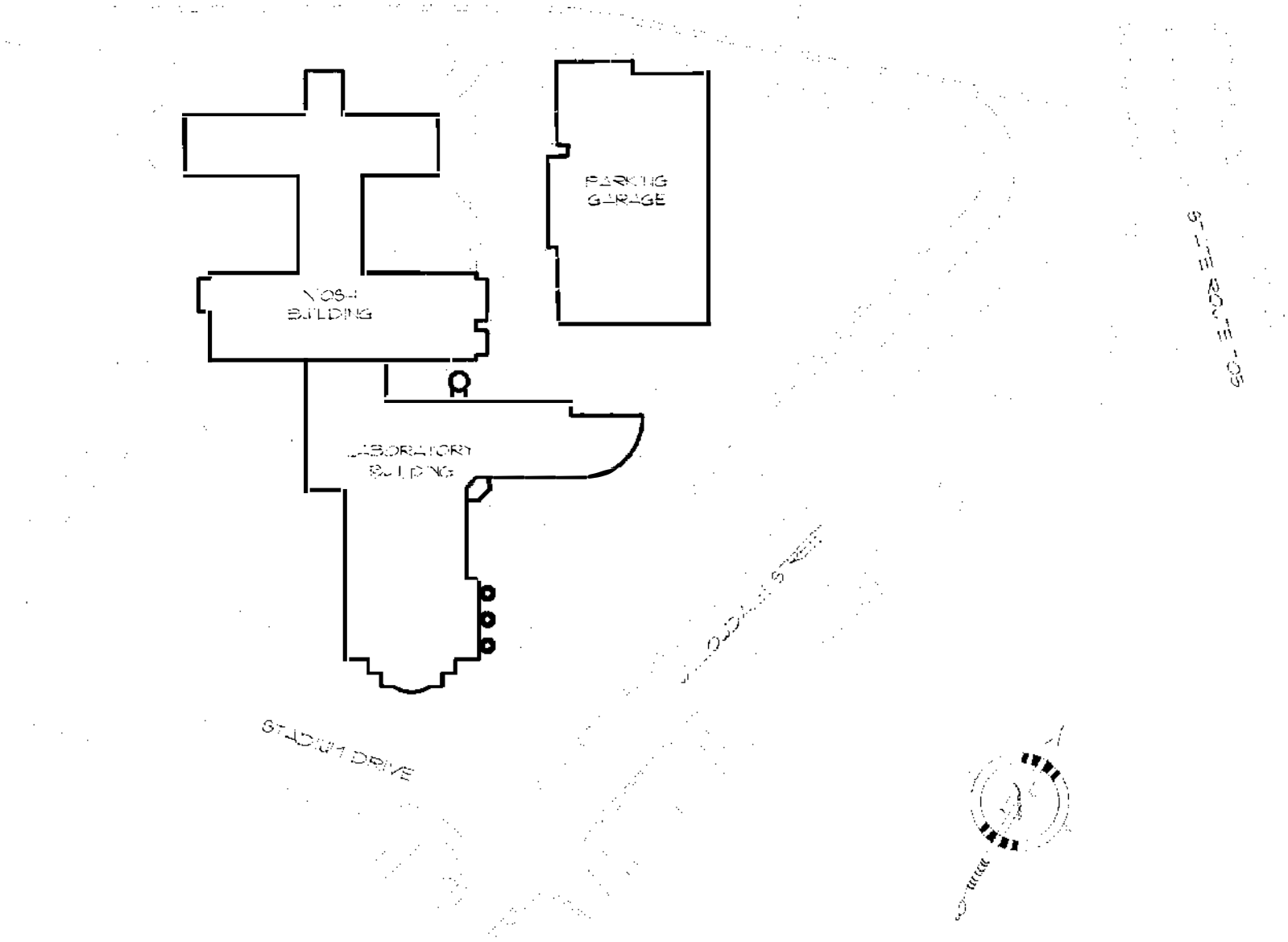


Appendix 3.1: Line Drawings
Animal and Inhalation Facility Space

Request that all line drawings showing identified room locations be redacted.

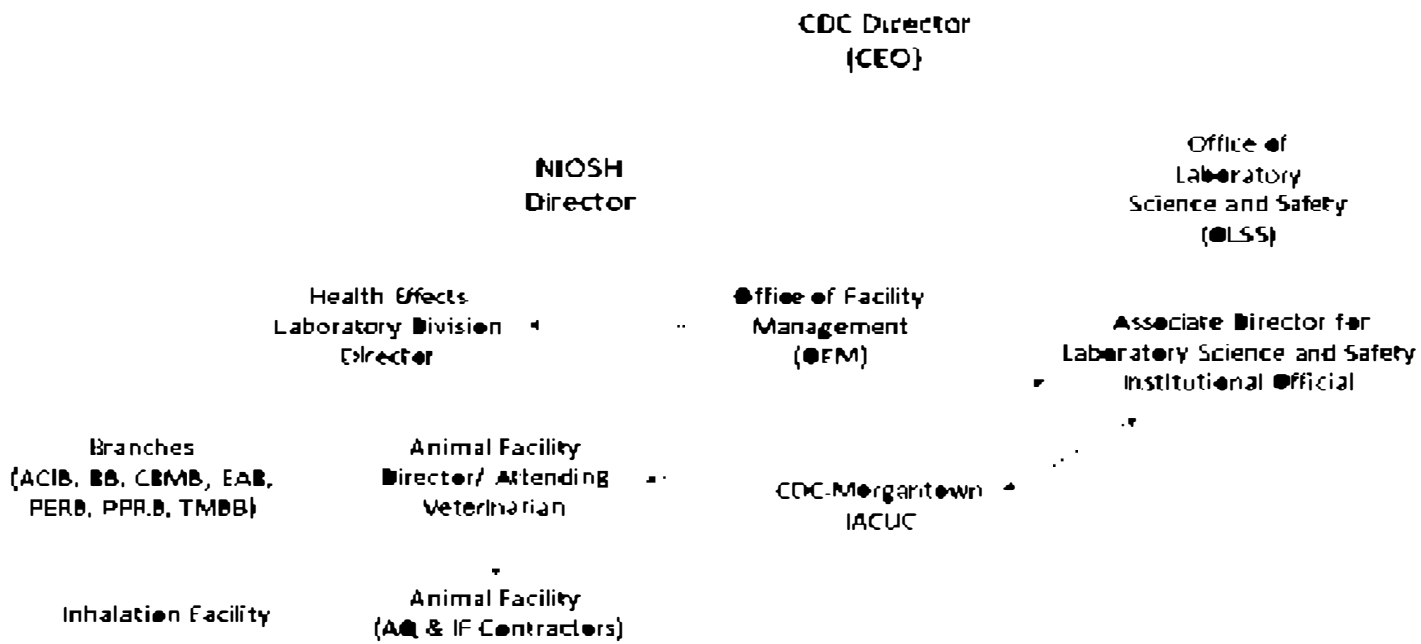
(b)(7)(E)

Appendix 3.2: Line Drawings
Site Map



Morgantown Campus

Appendix 4 – Organizational Chart



Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Electroencephalography (EEG) telemetry studies in rats to identify neurological risks associated with workplace chemicals & nanomaterials	(b)(7)(E)	(b)(6)	Rattus	88	D-38 E- 50	X				X	
Treatment screening in the Gulf War Illness mouse model			Mus	1590	C- 1030 E- 560					X	
Pulmonary function and MWCNT inhalation			Rattus	239	D					X	
Evaluation of the Hypersensitivity and Immunotoxicity of Commercially Available Products and their Components			Mus	285	C					X	
Exposure to stress hormones and neurotoxic agents as a model of Gulf War Illness (GWI) in mice: Expansion of the GWI Paradigm			Mus	1836	C- 730 E- 1106					X	
Exposure to stress hormones and the sarin surrogate, diisopropyl fluorophosphate (DFP), as a model of Gulf War Illness in mice			Mus	2018	C- 739 E-1279					X	
Minimally Invasive Biomarkers for Early Detection of Pulmonary Toxicity in Rats			Rattus	143	C					X	
Health Surveillance and Holding of Rats and Mice			Mus	1100	C						
Health Surveillance and Holding of Rats and Mice			Rattus	611	C						
NIOSH Breeding Program for Mice and Rats			Mus	15244	C- 15144 D-100						
NIOSH Breeding Program for Mice and Rats			Rattus	1124	C- 924 D-200						

Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Welding fume-related neurotoxicity in rats: Influence of shielding gases	(b)(7)(E)	(b)(6)	Rattus	96	C					X	
Mouse models of occupational lung disease with genetic modifications			Mus	598	D					X	
Conditional knockout mouse models of fibrosis			Mus	390	D					X	
Toxicological effects of inhaled silica used in hydraulic fracturing - Part 2			Rattus	30	C					X	
Exposure to Stress Hormones and the Sarin Surrogate, Diisopropyl Fluorophosphate (DFP), as a Model of Gulf War Illness in Rats.			Mus	66	C- 1030 E- 56					X	
Exposure to Stress Hormones and the Sarin Surrogate, Diisopropyl Fluorophosphate (DFP), as a Model of Gulf War Illness in Rats.			Rattus	792	C-330 E- 462					X	
Neurotoxicity and Neuroinflammation in Mice and Rats			Mus	40	C					X	
Neurotoxicity and Neuroinflammation in Mice and Rats			Rattus	10	C						
Age dependent adaptation/maladaptation in an in vivo mouse model			Mus	610	C- 300 D-310						
Pulmonary and Immune Effects Following Respiratory Exposure to Gold Nanoparticles			Mus	192	D					X	
Mechanistic studies of inflammation/carcinogenesis of different types of asbestos/asbestiform fibers and cleavage fragments.			Mus	720	D					X	
Exposomal and molecular markers of sensitization by dermal methylene diphenyl diisocyanate (MDI) sensitization.			Rattus	12	D					X	

Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Characterization of the exposome using a tiered, translational exposure assessment model	(b)(7)(E)	(b)(6)	Rattus	384	C					X	
Toxicological Investigations of Nitrogen-Doped Multi-Walled Carbon Nanotubes - Part 2			Mus	130	D					X	
A Toxicological Evaluation of Crystalline Nanocellulose Exposure in Mice			Mus	840	D					X	
Blood gene expression signatures to detect pulmonary toxicity induced by nanomaterials in rats			Rattus	288	C					X	
Cellular and Molecular Mechanisms of Immune Responses During Sub-chronic Exposure to Fungal Spores			Mus	875	D-172 E-703				X	X	
Mouse Models of Irritant- and Sensitizer-Induced Occupational Asthma			Mus	1176	D-876 E-300					X	
Pulmonary Toxicity and Epigenetic Effects of Metals found in Welding Fumes in Mice			Mus	241	D					X	
Exposome, metabolic dysfunction and silicosis: Cardio-pulmonary pathophysiology			Rattus	442	C-346 D-96					X	
Toxicity Associated with Boron Nitride Nanotube exposure			Mus	864	D					X	
Pulmonary Toxicity Assessment after Inhalation of a Copper-Nickel Welding Aerosol in A/J mice			Mus	160	D					X	
Nanoparticle Induced Lung Inflammatory, Fibrotic and Resolution Pathways			Mus	562	D					X	
Hazard Identification of Aerosolized 3D Printer Emissions			Rattus	186	C					X	

Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Inflammatory and fibrotic potential of machined nanoclay-enabled composite dust in mouse lung	(b)(7)(E)	(b)(6)	Mus	465	D					X	
Effects of Antimicrobial Chemical Exposure on Anti-Viral Immunity			Mus	110	D					X	
The immunological effect of antimicrobial chemicals on the skin microbiome			Mus	210	C						
Health effects of whole body vibration			Rattus	92	C- 8 E- 84				X		
Brown Norway Rat Exposure to Aerosolized 4,4'-Methylene Diphenyl Diisocyanate as a Model of Occupational Asthma			Rattus	27	B- 3 E- 24				X	X	
Effects of inhalation exposure to a peroxyacetic acid solution in mice			Mus	132	C-12 E-120					X	
Nanoparticle-Induced Neuropathology in Mice			Mus	196	D					X	
Pulmonary particle exposure and cardiovascular toxicity			Rattus	224	D	X				X	
Evaluation of a novel humanized mouse model to study the immunotoxicological effects of sensitizing metals			Mus	75	C						
Comparative pulmonary toxicities of fracking sand dusts in rats: pilot study			Rattus	110	D					X	
Antimicrobial exposure and novel mediators of occupational allergic disease			Mus	200	C						
Investigating Styrene-Induced Fixed Airways Disease in Mice			Mus	107	C- 5 D-102					X	

Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Mild traumatic brain injury (mTBI) in a rat model of projectile concussive impact	(b)(7)(E)	(b)(6)	Rattus	30	C-10 D-20						
The assessment of an organosilane-based coating of silica particles in the potential reduction of acute and subchronic lung toxicity			Rattus	96	D					X	
Toxicological effects of inhaled diesel exhaust: hydraulic fracturing - Part 2			Rattus	96	C					X	
Examining the role of type 2 innate lymphoid cells following repeated fungal inhalation exposures			Mus	450	E				X	X	
Obesity and Mouse Models of Occupational Asthma			Mus	864	D-432 E- 432					X	
Treatment screening in the Gulf War Illness mouse model			Mus	717	C- 297 E-420					X	
Exposure to stress hormones and acetylcholinesterase inhibitors as a model of Gulf War Illness in mice			Mus	824	C- 334 E- 490					X	
Exposure to stress hormones and acetylcholinesterase inhibitors as a model of Gulf War Illness in mice: evaluating the role of glia			Mus	469	C- 147 E- 322					X	

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

(2) Survival Surgery (SS)

(3) Multiple Survival Surgery (MSS) – Not applicable

(4) Food or Fluid Regulation (FFR)– Not applicable

(5) Prolonged Restraint (PR)

(6) Hazardous Agent Use (HAU)

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

Pain/Distress Classification Description/Definition, if applicable: USDA definitions are used at this time.

Appendix 5.1: Animal Usage Protocol Summary

July 2020 Update to Appendix 5.1: Animal Usage Protocol Summary

Protocol Title	Protocol #	Principal Investigator	Genus	Total Number of Animals Approved	Pain & Distress Category	SS (2)	MSS (3)	FFR (4)	PR (5)	HAU (6)	NCA (7)
Small molecule biomarker discovery for nanomaterial exposure and adverse outcomes	(b)(7)(E)	(b)(6)	*Rattus*	355	D = 355					X	
Resolution of lung inflammation in mice exposed to fibrogenic particles and nanotubes			*Mus*	474	C = 12 D = 462					X	
The role of non-pathogenic cryptococcus species exposure on the mediation of airway immune response against ovalbumin			*Mus*	172	D = 172					X	
Toxicity assessment of metal oxide mixtures found in welding fumes			*Mus*	576	D = 576					X	
The effect of welding fume exposure on crystalline silica-induced pulmonary toxicity			*Rattus*	144	C = 144					X	
Toxicological effects of inhaled fracking sand dust together with diesel exhaust: hydraulic fracturing			*Rattus*	528	C = 288 D = 240	X				X	
Health surveillance and holding of rats and mice			*Mus* *Rattus*	904 745	C- 604 D-300 C = 445 D = 300						
Evolution of the subchronic toxicity of *Aspergillus versicolor* in B6C3F1/N mice exposed via nose-only inhalation			*Mus*	80	E = 80				X	X	
Rodent Breeding Program			*Mus* *Rattus*	11400 1000	C= 11200 D= 200 C= 880 D= 120	X					
The toxicity and associated health outcomes following dermal exposure to Per- and Polyfluoroalkyl Substances (PFAS)			*Mus*	48	C = 48					X	
Characterization of KOLC conditional knockout mouse model of fibrosis			*Mus*	312	D = 312					X	

Appendix 5.1: Animal Usage Protocol Summary

Age dependent (muscle) adaptation/maladaptation in an *in vivo* mouse model	(b)(7)(E)	(b)(6)	***Mus***	**348**	**C = 174 D = 174**							**X**

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

(2) Survival Surgery (SS)

(3) Multiple Survival Surgery (MSS) – Not applicable

(4) Food or Fluid Regulation (FFR)– Not applicable

(5) Prolonged Restraint (PR)

(6) Hazardous Agent Use (HAU)

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

Pain/Distress Classification Description/Definition, if applicable: USDA definitions are used at this time.

Appendix 5.2: Animal Usage

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

Animal Type or Species	Approximate Annual Use
Mus musculus	7,400
Rattus norvegicus	1,440
Caviae porcellus	6

Appendix 6.1: Personnel Medical Evaluation Form
NIOSH Personnel

Laboratory Animal Allergy Questionnaire

Date _____

Name: _____

Supervisor: _____

Department: _____

Age: _____ Sex: ____Male ___Female

OCCUPATIONAL HISTORY
Answer these questions about your present job:
Job title: _____
Number of years employed at this facility: _____
How many months/years at your present position? _____

Brief description of duties

_____
_____
_____

Do you work with laboratory animals? ___Yes ___No
If yes, complete the following.

Animal	Yes	No	Approximate Contact Hours/Day
Rats	___	___	_____
Mice	___	___	_____
Rabbits	___	___	_____
Guinea Pigs	___	___	_____
Other	___	___	_____

Do you feel that you are allergic to any of these animals? ____Yes ____No

___ Rats	___ Mice	___ Rabbits	___ Dogs	___ Other
___ Cats	___ Monkeys	___ Cattle	___ Guinea Pigs	

Did you work with laboratory animals before your employment at this facility?
___ Yes ___No
If yes, how long? ___ ___years What type of animals? _____

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

Protective Eye Glasses	___Yes ____No
Mask/Respirator	___Yes ____No
Lab Coat	___Yes ____No
Gloves	___Yes ____No

HOME ENVIRONMENT INFORMATION
Do you have any indoor pets? ____Yes ____No If yes, which animals and for how long?

Animal	1-2 Years	2-3 Years	3-4 Years	Over 4 Years
Dogs	___	___	___	___
Cats	___	___	___	___
Other (Type)___	___	___	___	___

Do you regularly have any of the following symptoms? ____Yes ____No Please indicate if the symptom is present and the year of onset. Also check in what location or time "period" the symptom(s)is/are present.

Appendix 6.1: Personnel Medical Evaluation Form
NIOSH Personnel

Symptom	Yes/No Present	Year of Onset	Symptoms Are Present At Work	At Home	On Vacation	No Difference
Cough	___	___	___	___	___	___
Sputum Production	___	___	___	___	___	___
Shortness of Breath	___	___	___	___	___	___
Wheezing	___	___	___	___	___	___
Chest Tightness	___	___	___	___	___	___
Asthma	___	___	___	___	___	___
Nose Congestion	___	___	___	___	___	___
Runny Nose	___	___	___	___	___	___
Sneezing	___	___	___	___	___	___
Itchy Eyes	___	___	___	___	___	___
Sinus Problems	___	___	___	___	___	___
Hay Fever	___	___	___	___	___	___
Frequent Colds	___	___	___	___	___	___
Hives	___	___	___	___	___	___
Skin Rash	___	___	___	___	___	___
Swelling of Eyes or Lips	___	___	___	___	___	___
Eczema	___	___	___	___	___	___
Difficulty in Swallowing	___	___	___	___	___	___

Were you ever told by a doctor that you had allergies?___Yes___No

Have you ever been skin tested for allergies?____Yes____No If yes, what substances were you found to be allergic to or sensitized to?

___ Ragweed___Grass____Trees____Mold____Dust____Cat____Dog____Mice ____

Other _____

Have you ever received allergy(desensitization/immunotherapy) shots?____Yes____No

Has a doctor ever said you have asthma?____Yes____No

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

Are you currently taking medication (either over the counter or by prescription) to control your asthma?
___ Yes___No

Has a doctor ever told you that you have a medical condition caused by your working conditions?
___ Yes___No

Do any of your blood relatives (grandparents, parents, brothers/sisters) have allergies or asthma?
___ Yes___No

Are you under a doctors care for any other illnesses?
___ Yes___No If

yes, please list illnesses:

_____

_____

Do you take blood pressure medication(s)?___Yes___No

Do you regularly use "over the counter" (nonprescription) nose drops or nose sprays (e.g., Afrin, Neosynephrine)?____Yes___No

Do you smoke cigarettes?____Yes___No If yes, how many cigarettes per day?_____

Appendix 6.1: Personnel Medical Evaluation Form
NIOSH Personnel

How many years?_____

If not presently smoking, did you ever smoke?____Yes____No If

yes, when did you stop smoking cigarettes?_____(year)

How many years did you smoke?_____years Comments

Appendix 6.1: Personnel Medical Evaluation Form
NIOSH Personnel

Medical recommendation

Employee _____

☐ The employee is cleared for contact with the research animals.

☐ The employee is cleared for contact with the research animals with the following restrictions:

_____

_____

_____

☐ The employee is not cleared for contact with the research animals.

Reviewed By:_____Date:_____

Appendix 6.2 Personnel Medical Evaluation Form
Contract Personnel in Animal & Inhalation Facility

(b)(4)

Appendix 6.2 Personnel Medical Evaluation Form
Contract Personnel in Animal & Inhalation Facility

OCCUPATIONAL HEALTH QUESTIONNAIRE

CONFIDENTIAL: FOR MEDICAL USE ONLY

Today's date: _____

*Name:*_____ *Sex:*_____*Birthdate:* _____

*Job Title or Category:*_____ *Years in present position:* ____

*Phone no:*_____ *Marital Status:* ___

Brief description of duties: _____

_____

A. Please answer the following questions by checking the applicable blocks:

Have you ever been hospitalized? NO ☐ YES ☐

*If yes, give details and dates:*_____

_____

*Indicate any occupational illnesses or injuries you have experienced since being employed by Healtheon, Inc.:*_____

_____

_____

Have you ever been a resident outside the United States: NO ☐ YES ☐

*If yes, please list location(s) and date(s):*_____

_____

Have you ever served in the armed forces or merchant marines? NO ☐ YES ☐

*If yes, indicate geographic areas of duty and dates:*_____

_____

*Please make a list of those substances that you now handle in your work, hobbies, or pastime. Star (*) those that particularly concern you from a health standpoint:*_____

_____

_____

*Have you ever worked with any other substance which you think might be or may have been hazardous (please list)?*_____

_____

_____

*Do you have or have you made any suggestions to reduce potential exposures?*_____

_____

_____

*Indicate any signs or symptoms that you have or have experienced that might be due to exposure at work and indicate the suspected cause:*_____

B. Do you have or have you had any of the following? If so, please check the appropriate boxes.

- ☐ allergies
- ☐ back pain
- ☐ blood in urine, sputum or stool
- ☐ blood pressure changes
- ☐ bowel changes
- ☐ cancer
- ☐ chest pain
- ☐ chronic cough
- ☐ cold or painful fingers
- ☐ dental or gum problems
- ☐ depression or excessive worry
- ☐ diabetes
- ☐ diarrhea
- ☐ difficulty in sleeping
- ☐ dizziness
- ☐ ear or hearing problems
- ☐ edema (feet or legs swelling)
- ☐ eye trouble (other than corrective lenses)
- ☐ fainting spells or unconsciousness
- ☐ fever of unknown origin
- ☐ frequent indigestion or stomach trouble
- ☐ frequent or severe headaches
- ☐ heart problems (fast beat, murmurs, pain, etc.)
- ☐ hemorrhoids (piles)
- ☐ hives or skin rash
- ☐ hot flashes or flushing
- ☐ joint pains
- ☐ kidney problems
- ☐ leg cramps
- ☐ liver problems
- ☐ loss of or poor memory
- ☐ lung problems or breathing difficulty
- ☐ menopause symptoms
- ☐ muscle aches or pains
- ☐ nervousness or irritability
- ☐ sexual problems
- ☐ sickle cell disease or trait
- ☐ skin disease or problems
- ☐ swollen glands (groin, underarm, neck)
- ☐ thyroid gland problem
- ☐ tremor of hands or head
- ☐ tumors, cysts, or mass
- ☐ unexpected weight changes
- ☐ unusual weakness or fatigue
- ☐ venereal disease

C. Family History
Indicate any blood relatives that you know of who ever had any of the following:

Appendix 6.2 Personnel Medical Evaluation Form
Contract Personnel in Animal & Inhalation Facility

Disease	*Relationship to You*				
	Mother	*Father*	*Grandparent*	*Brother/ Sister*	*My children*
Anemia	☐	☐	☐	☐	☐
Arthritis	☐	☐	☐	☐	☐
Allergy (asthma, eczema, hay fever)	☐	☐	☐	☐	☐
Alcoholism	☐	☐	☐	☐	☐
Bleeding disorders	☐	☐	☐	☐	☐
Congenital malformations or abnormalities	☐	☐	☐	☐	☐
Cancer	☐	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐	☐
Emphysema	☐	☐	☐	☐	☐
Epilepsy	☐	☐	☐	☐	☐
Glaucoma (incl. Eye pressures)	☐	☐	☐	☐	☐
Gout or painful joints	☐	☐	☐	☐	☐
Heart problems	☐	☐	☐	☐	☐
High blood pressure	☐	☐	☐	☐	☐
Disease	*Mother*	*Father*	*Grandparent*	*Brother/ Sister*	*My children*
Kidney disease or stones	☐	☐	☐	☐	☐
Mental problems	☐	☐	☐	☐	☐
Sickle cell disease or trait	☐	☐	☐	☐	☐
Stomach problems	☐	☐	☐	☐	☐
Stroke	☐	☐	☐	☐	☐
Thyroid conditions	☐	☐	☐	☐	☐
Tuberculosis	☐	☐	☐	☐	☐
Other (specify)	☐	☐	☐	☐	☐

D. Please indicate the length of exposure time to the following types of laboratory animals in your work:

Animal	Yes	No	Approximate Contact Hours/Day
Rats	___	___	_____
Mice	___	___	_____
Guinea Pigs	___	___	_____

What animal(s) do you feel you are allergic to?

___ Rats ___ Mice ___Rabbits ___ Dogs ___Other
___ Cats ___ Monkeys___Cattle ___ Guinea Pigs

Did you work with laboratory animals before your employment at this facility?

___ Yes___No

If yes, how long?_____years What type of animals? _____

Do you use or wear any of the following items when working with animals?
Protective Eye Glasses ___Yes____No
Mask/Respirator ___Yes____No
Lab Coat ___Yes____No
Gloves ___Yes____No

HOME ENVIRONMENT INFORMATION
Do you have any indoor pets?____Yes____No If yes, which animals and for how long?

Animal	1-2 Years	2-3 Years	3-4 Years	Over 4 Years
Dogs	___	___	___	___
Cats	___	___	___	___
Other (Type)_____	___	___	___	___
_____	___	___	___	___

Were you ever told by a doctor that you had allergies?____Yes____No

Have you ever been skin tested for allergies?____Yes____No If yes, what substances were you found to be allergic to or sensitized to?

___ Ragweed___Grass___Trees___Mold___Dust___Cat___Dog _ Mice ___

Other _____

Have you ever received allergy (desensitization/immunotherapy) shots?____Yes____No

Has a doctor ever said you have asthma?____Yes____No

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

Are you currently taking medication (either over the counter or by prescription) to control your asthma? ___Yes____No

Has a doctor ever told you that you have a medical condition caused by your working conditions?
___ Yes___No

Comments

_____

_____

E. If either of your parents are dead list their age at death and cause of death, if known:

mother died at age___of _____
father died at age____of_____

Are you under a doctor's care for any other illnesses?
NO ☐ YES ☐

If yes, please list illnesses:

_____

_____

F. During a typical week, on approximately how many days do you drink alcoholic beverages?
_____days *(mark 0 if you do not drink alcoholic beverages)*

If you drink, do you ever drink on those days when you work?
NO ☐ YES ☐

If "yes," do you normally drink....?
☐ before going to work
☐ after finishing work
☐ during lunch breaks
☐ during rest breaks when at work

On those days when you do drink, about how many of each of the following do you usually drink? (mark 0 if you do not drink alcoholic beverages.):

_____bottles of beer_____glasses of wine_____shots of liquor (shot = 1 1/2 oz.)

Would you say that the amount of alcohol have been drinking lately has...?
☐ increased ☐ decreased ☐ remained about the same ☐ don't drink

Would you say that you use alcohol to help you get to sleep...?
☐ frequently ☐ occasionally ☐ seldom ☐ never

G. Do you smoke: NO☐ YES ☐

If no, are you a former smoker? NO ☐ YES ☐

*If yes, how long ago did you quit?*_____years

How many years did you smoke before quitting? _____years

How much were you smoking before you quit? That is, number of cigarettes, cigars, or pipes smoked per day? _____

If yes, please answer the following:

*How long have you smoked?*_____years

How many of the following do you smoke per day?

_____cigarettes _____pipefuls of tobacco _____cigars

Would you say that your amount of smoking lately has . . .? (check one)
☐ increased ☐ decreased ☐ remained the same

H. What time do you go to bed during the week? _____
What time do you go to bed on the weekend? _____
What time do you awake during the week? _____
What time do you awake on the weekend? _____

I. Have you experienced any problems in the last year with feeling depressed, solemn, or otherwise unhappy over a long period of time? If so, please explain:

_____
_____
_____
_____

J. Females only *

Number of normal pregnancies*_____Number of living children* _____

**Number of involuntary miscarriages* _____

**Date of last mammogram* _____

**Date of last pap test*_____ **Date of last period* _____

**Have you had any unusual discharge or bleeding in the past three months?*
NO ☐ YES ☐

**Have you had any unusual discharge or bleeding at any time?* NO ☐ YES ☐

**Have you reached menopause?* NO ☐ YES ☐

Have you or your present or former spouse had any adverse reproductive outcome? For example: stillborn, deformed, irregular menses? NO ☐ YES ☐

K. Medication History

List medications you take regularly:

_____
_____

L. *Have you been on any diet(s) in the past year?* NO ☐ YES ☐
if yes, describe type (for overweight, underweight, diabetic, etc.):

M *Immunization vaccines antitoxins etc.*

Check if you have received any of the following. Give approximate date(s) when last received, if known:

	Dates
☐ tetanus	
☐ poliomyelitis	
☐ influenza	
☐ typhoid	
☐ diphtheria	
☐ rabies	
☐ rabies	
☐ rubella (german measles)	
☐ measles (rubella or red measles)	
☐ bcg	
☐ yellow fever	
☐ anthrax	
☐ small pox	
☐ rhogam (rh immune globulin)	
☐ immune serum globulin (for hepatitis)	
☐ others (please list)	
☐ mantoux, patch test, or other skin test for tuberculosis. Give date and result of first test, if known	Date Result: ☐ positive ☐ negative

When you have finished this medical and occupational history form, hand it directly to the doctor or nurse, or if mailed, mark envelope "to be opened by medical personnel."

Appendix 7: IACUC Roster

IACUC Roster, Updated July 2020

Name	ACUC Role	Branch	Degree/ Credentials
(b)(6)	Scientist, Chair	ACIB	PhD
	Scientist, Alternate	ACIB	PhD
	Scientist, Alternate	ACIB	PhD
	Scientist, ACUC Administrator	HELD/OD	MS
	Attending Veterinarian	HELD/OD	DVM
	Non-affiliated / Non-Scientist	NA	BS
	Member, Alternate Safety	OFM	MS
	Member, Safety	OFM	MS
	Scientist	PERB	PhD
	Scientist	PPRB	PhD
	Scientist, Alternate	PPRB	PhD
	Scientist	PPRB	PhD
	Scientist	TMBB	PhD
	Scientist, Alternate	TMBB	MS
	Administrator, Scientist, Alternate	TMBB	BS
	Scientist, Vice Chair	TMBB	PhD

Meeting Minutes
Animal Care and Use Committee
September 19, 2019
(b)(7)(E) @ 9:00 a.m.

Members present: (b)(6)
(b)(6)

Absent: (b)(6)

Guest: (b)(6)

The meeting was called to order at 9:00 a.m. and was adjourned at 10:35 a.m.

Old Business:

Meeting Minutes

Minutes of August 15 Meeting– Approved as written

Previous Protocol Reviews

(b)(7)(E) (b)(6) – Full Committee Review 08/16/2018; Comments sent to PI 08/17/2018; PI response 11/27/2018; Under DMR; Returned to PI 04/04/2019

(b)(7)(E) (b)(6) - Full Committee Review 11/15/2018; Comments sent to PI 11/20/2018

(b)(7)(E) (b)(6) – Full Committee Review 08/15/2019; Comments sent to PI 08/15/2019; PI response 08/27/2019; Under DMR; **Approved 09/03/2019**

New Business:
(Adverse Events, Compliance Issues, Post Approval Monitoring, Animal SOPs, Committee Membership, etc. addressed in the following reports)

Chair Report

Conflict of Interest and Confidentiality Reminder
CDC-Atlanta IACUC Meeting- attended 09/09/19; points to consider/ adapt for CDC-Morgantown IACUC- review/ modify CDC-Atlanta policies for our animal program, include short training section in out monthly IACUC meetings and include more detail in the meeting minutes
Adverse Event Follow up – (b)(7)(E) (b)(6) - memo sent to IO, 08/12/19; memo sent to PI, 08/12/19; IO Memo/ Report sent to OLAW, 09/16/19
Adverse Event Follow up – (b)(7)(E) (b)(6) – memo reporting event sent to ACUPO/ IO, 09/09/19

IACUC Administrator Report

Fall Semiannual Inspection date & program review - 10/3,10/7- 10/9; revised inspection forms for AF will be emailed to AV & AF Mgr. the wk. of 09/16; revised forms for labs will be emailed to PIs the wk. of 09/23; new SOP-OD-00346 for the Handling and Use of Controlled Substances (Schedule I and II) will be attached to PIs' email
FY2019 Annual Review of Animal Use- 1st email notification to PIs, 09/30
IACUC Inter-Institutional/ Collaborative Agreement – edited version returned to ACUPO for comments, 07/31/19; sent follow-up email, 09/16/19; ACUPO response, 09/17/19- will review soon
CDC-Morgantown IACUC Charter- submitted to CDC/OLSS/OD for re-formatting, 05/09/19; requested status update, 06/21/19; OLSS reply, 06/21/19; requested status update, 09/16/19; OLSS reply, 09/18/19; OLSS plans to schedule a Skype meeting with IACUC Chair, IA and AV the week of 09/23/19
IACUC Administrator back-up- (b)(6) in attendance; has begun working with IA to learn IA responsibilities & work activities with the goal of assuming role of IA by the end of CY 2020

Attending Veterinarian/ Facility Director Report

Animal census numbers- animal numbers remaining stable
AF Working Standards – 3 approved: H-01- Receipt of Scheduled Feed and Bedding Deliveries, H-02- Receiving Animal Deliveries from Commercial Vendors & H-03- Cage Access for Whole Body Inhalation Studies: Rats in Room (b)(7)(E) and Mice in (b)(7)(E) 1 in review: E-01- Use of Carbon Dioxide (CO2) for Euthanasia of Mice and Rats using the Euthanex brand SmartBox® Fixed Flow (SBFF) Auto CO2 System; 1 in draft/ revision: G-01- Personal Protective Equipment (PPE) and Clothing Requirements for the Animal Facility
PHS OLAW Animal Welfare Assurance for Domestic Institutions (Domestic Assurance) –submitted to OLAW 8/31/19
Façade renovation (Oct- Dec 2019)- may have effect on IF exposure studies
CO2 Euthanasia - CO2 - AIMS Flow Meter euthanasia system will be relocated in (b)(7)(E) (Histopathology Core Lab); CO2 - AIMS Flow Meter euthanasia system located in (b)(7)(E) will be updated with cages and Euthanex lids
One IF technician has left (09/13); AF Manager has been interviewing candidates for position

Program

Adverse Events- (b)(7)(E) (b)(6) – IACUC members voted that additional details of the adverse event are required before deciding if the event is reportable to OLAW and if further action is required; the IACUC Chair will contact the PI and will provide additional details during the October IACUC Meeting

Post Approval Monitoring (PAM)

(b)(7)(E) (b)(6) – performed 09/09/19

PAM summary- Post-approval monitoring (PAM) was conducted on September 9th, 2019 for the purposes of executing the IACUC request for PAM to observe the set up and function of the gas anesthesia and CPI (cranial percussive injury) apparatus and to observe the recovery and neurological deficits of post-injury rats. Three groups of Sprague Dawley rats (control, aluminum, and stainless steel) were evaluated at 2 timepoints (6 hours and 24 hours) in this pilot study.

IACUC discussion- All animals in the control (blast of air) and aluminum groups seemed to recovery well after the exposure and obtained normal scores. The effect from the stainless steel were remarkably more severe and quite variable with two deaths. The investigators necropsied the two that died and detected no cranial fractures, but both had substantial intra-cranial bleeding around the brain. It was determined that the animals in the air control could be Category D while the aluminum and stainless steel animals need to remain Category E. Additionally, the committee felt that the investigators need to determine the force at which the animal's skull is being impacted, especially in the case of the stainless steel ball. Additional factors the investigators need to consider before additional studies will be approved are: the speed of the projectile, how to consistently identify the point of contact of the projectile on the head at impact, and consistent alignment of the animal when placed in the testing apparatus prior to initiating the projectile. Photographing exposures is also recommended to better define the force being projected by both type balls. The inclusion of a pilot study (no animals) using high speed camera to measure and calculate a range of accelerations produced when the aluminum and stainless steel balls are shot from the apparatus is also recommended.

NIOSH Breeding Program

Breeding colonies are producing well

Upcoming IACUC 101/201/301 meetings

November 6, 7, 8, 2019: IACUC 101, 201 and 301 in Houston, TX - Hosted by Rice University

Reports:

Investigators trained or re-trained:

NONE

ACUC SOPs reviewed:

SOP-OD-00346 for the Handling and Use of Controlled Substances (Schedule I and II)– approved (8/15/19)

SOPs nearing expiration (120 days):

NONE

Amendments: Records of reviews by e-mail are kept with protocol files.

(b)(7)(E) (b)(6) - Rec'vd 04/04/19; Under DMR; Comments sent to PI 04/09/19;
(b)(7)(E) (b)(6) - Rec'vd 04/09/19; Under DMR; Comments sent to PI 04/15/19; PI response 04/22/19; Under DMR; Comments sent to PI 05/01/19
(b)(7)(E) (b)(6) - Rec'vd 06/21/19; FCR requested 06/24/19; FCR 07/18/19; Comments sent to PI 07/19/2019; **Withdrawn 08/15/19**
(b)(7)(E) (b)(6) - Rec'vd 08/16/19; **Approved by VVC 08/21/19**
(b)(7)(E) (b)(6) - Rec'vd 08/19/19; Under DMR; **Approved 08/28/19**

Protocols that have expired:

NONE

Protocols nearing expiration (120 days): (Investigators have been notified.)

(b)(7)(E) (10/20/2019)
(11/21/2019)
(11/16/2019)

(b)(7)(E) (10/28/2019)
(11/21/2019)
(12/23/2019)

Protocols closed at request of PI:

NONE

Full-Committee Reviews:

(b)(7)(E) (b)(6) – lengthy discussion regarding ozone level used for animal exposures and frequency of animal monitoring during ozone exposure experiments- IACUC opinion is that the animals need to be checked in 10 - 15 minute intervals vs the 30 minute interval proposed by the PI; unanimous approval to DMR
CDC-Morgantown IACUC Policy P-008 for Post-Approval Monitoring (PAM)- approved with minor edits
IACUC P-0008 Addendum 1: PAM Observation Form- approved as written

Topics for Discussion

Next Meeting:

Next Meeting – Thursday, October 24, 2019 at 9:00 a.m. in (b)(7)(E)

Meeting Minutes
Animal Care and Use Committee
October 24, 2019
(b)(7)(E) 9:00 a.m.

Members present (b)(6)
(b)(6)

Absent: (b)(6)

Guest: (b)(6)

The meeting was called to order by Vice Chair (b)(6) t 9:00 a.m. and was adjourned at 11:55 a.m.

Old Business:

Meeting Minutes

Minutes of September 19 Meeting– Approved as written

Previous Protocol Reviews

(b)(7)(E) (b)(6) – Full Committee Review 08/16/2018; Comments sent to PI 08/17/2018; PI response 11/27/2018; Under DMR; Returned to PI 04/04/2019
(b)(7)(E) (b)(6) - Full Committee Review 11/15/2018; Comments sent to PI 11/20/2018
(b)(7)(E) (b)(6) – Full Committee Review 09/19/2019; Comments sent to PI 09/20/2019; PI response 10/08/2019; Under DMR; **Approved 10/15/2019**

New Business:
(Adverse Events, Compliance Issues, Post Approval Monitoring, Animal SOPs, Committee Membership, etc. addressed in the following reports)

Chair Report

Conflict of Interest and Confidentiality Reminder
Adverse Event Follow up - (b)(7)(E) (b)(6) – PAM performed, 10/22/19
Adverse Event Follow up - (b)(6)) – IO sent report to OLAW, 10/02/19
Semi-annual Inspection of Labs/ Animal Facility- sending the inspection forms out ahead of time to the PIs to fill out before the inspection reduced the time of the inspection process; the Spring Semi-annual Inspection will be scheduled for 2 days (1 day for all labs and 1 day for the animal facility) instead of the current 4 days; noted that there still seems to be confusion about what are 'child bottles' for (b)(7)(E)

IACUC Administrator Report

Fall Semiannual Inspection- 10/3, 10/7-10/9; revised inspection forms for AF and laboratories will be further revised as a result of the inspection to improve/clarify questions and information gathered; inspection findings- mostly minor findings; labs- IACUC notebook recordkeeping; key t(b)(7)(E) (b)(7)(E) in 1 lab; animal facility - 2 bottles of expired antibiotic in 2 locations (immediately removed); expired disinfectant (removed/ replaced) in several locations
FY2019 Annual Review of Animal Use- due back to IA, 10/31/19; IA provided animal numbers to PIs due to Topaz software issue (PIs couldn't access the information for their specific protocols); to date- 37/63 reports reviewed & approved; 4/63 reports need revised; 22/63 reports outstanding.
IACUC Inter-Institutional/ Collaborative Agreement – no update
CDC-Morgantown IACUC Charter- submitted to CDC/OLSS/OD for re-formatting, 05/09/19; IACUC Chair, Administrator, and Attending Veterinarian had two conference call with OLSS IACUC Liaison 9/30 & 10/18; Charter will be edited to reflect our suggestions, forwarded to IO

Attending Veterinarian/ Facility Director Report

AALAS Meeting (Denver, CO), 10/13- 10/17/ 2019 Summary- Protocol Annual Reviews may not be required next year; USDA & OLAW annual report dues will soon coincide; EPA director initiative to reduce animal research 30% by 2025 and completely eliminate it (with loophole for exceptions) by 2035; 2 new (rare) viruses discovered; another mouse adenovirus and rat polyoma virus 2- IDEXX is developing tests for these.
Animal census numbers- steady but low, and not seeing a new FY surge yet.
AF Working Standards – (b)(6) working with AF Manager & AV in updating & reformatting working standards
Façade renovation (Oct- Dec 2019)- may have effect on IF exposure studies and breeding colony production. This remains a concern, but lately has made much less noise than expected. Vibration recording conducted two weeks ago, but two of the data loggers are missing.

Program

Semi-annual Program Review: FCR of CDC-IACUC Policies P-0001(Social Housing); P-0003 (Euthanasia); P-0004 (Conflict of Interest); P-0005 (Satellite Animal Facility) – IACUC approved revisions suggested by AV to P-0003 on Euthanasia regarding cervical dislocation; also look into training and proficiency assessment for cervical dislocation. After discussion of P-0005 on Satellite Animal Facility- the AV will further review, discuss with Chair and IA, and bring suggested revisions, or recommendation to not revise at this time, to the November IACUC Meeting. Need to determine if (b)(7)(E) should be considered a Satellite room, consider relaxing some room (macroenvironment) standards and whether and with what conditions to reopen the (b)(6) lab room(s) (b)(7)(E) with IVC housing for short period use.

Adverse Events-

Follow-up: (b)(7)(E) (b)(6) – Post-Approval Monitoring performed, 10/22/2019

Post Approval Monitoring (PAM)

(b)(7)(E) (b)(6) – performed 09/09/19
PAM summary- Post-approval monitoring (PAM) was conducted on October 22, 2019 for the purposes of executing the IACUC request for PAM to observe the PI performing the restraint of mice in Broome restrainers and nail nick procedure. However, on this day the procedures were performed by a post-doctoral fellow (b)(6) and a graduate student (b)(6). The PAM committee was unable to observe the PI perform the procedure. The PI indicated he is generally involved in performing these procedures when one or more lab members are not available.

Observation: The procedure was very well organized; (b)(6) are clearly proficient with the restraint and tail-nick procedure.

IACUC discussion- The committee concluded that the investigation into the adverse event cannot be closed until the PI is observed performing the restraint procedure that initiated the Adverse Event Report and the subsequent IACUC investigation & PAM.

NIOSH Breeding Program

Discussion of 55% relative humidity improving breeding, as reported in seminars at the National AALAS meeting. Assessing levels of vibrations caused by the building façade renovation and being vigilant for possible effects on the breeding colonies; plan to move one breeding colony from an outer room to a inner room in the animal quarters. Looking for short term money (10K or so) for installing light timer alarms in the AF rooms. May consider installation of blue LED lights for the major HVAC controls renovation, which we hope will begin in about 1 year.

Upcoming IACUC 101/201/301 meetings

November 6, 7, 8, 2019: IACUC 101, 201 and 301 in Houston, TX - Hosted by Rice University

Reports:

Investigators trained or re-trained:

NONE

ACUC SOPs reviewed:

NONE

SOPs nearing expiration (120 days):

SOP-OD-00314 (exp. 01/26/2020)- Mouse oropharyngeal aspiration

Amendments: Records of reviews by e-mail are kept with protocol files.

(b)(7)(E) (b)(6) - Rec'vd 04/04/19; Under DMR; Comments sent to PI 04/09/19

(b)(7)(E) (b)(6) - Rec'vd 04/09/19; Under DMR; Comments sent to PI 04/15/19; PI response 04/22/19; Under DMR; Comments sent to PI 05/01/19

(b)(7)(E) (b)(6) - Rec'vd 10/08/19; Under DMR; **Approved 10/18/19**

(b)(7)(E) (b)(6) - Rec'vd 10/15/19; Under DMR; Comments sent to PI 10/22/19; PI response 10/22/19

(b)(7)(E) (b)(6) - Rec'vd 10/16/19; Under DMR; Comments sent to PI 10/23/19; PI response 10/24/19

(b)(7)(E) (b)(6) - Rec'vd 10/18/19; Under DMR

(b)(6) - Rec'vd 10/21/19; request for FCR 10/24/19

Protocols that have expired: (3 Legacy protocols remaining for CY2019 expiration)

(b)(7)(E) (10/20/2019)

Protocols nearing expiration (120 days): (Investigators have been notified.)

(b)(7)(E) (10/28/2019)
(11/21/2019)
(12/23/2019)

(b)(7)(E) (11/21/2019)
(11/16/2019)

Protocols closed at request of PI:

(b)(7)(E) (10/20/2019)
(12/21/2020)

(b)(7)(E) (7/11/2020)

Full-Committee Reviews:

(b)(6) 1. Committee discussed at length the roles and responsibilities of the protocol associates pertaining to euthanasia methods, particularly by focused-beam microwave irradiation. Only two associates are well trained in this procedure. Committee suggested removing this task from associates not trained in this procedure.
Alternatively, PI needs to provide adequate training to all associates, after which a PAM is to be done to ensure the procedure is conducted properly by the newly trained associates. 2. Suggested expansion of the duplication search strategy to include AChE inhibitors & the treatments (drugs) proposed in the protocol. 3. Suggested providing drug dose ranges and routes of exposures. 4. The anticipated 50% mortality rate was discussed and a decision was made to have sub- committee (consisting of the Chair, Administrator, primary reviewer, secondary reviewer) to meet with PI and/or lab members to discuss the mortality issues. 4. Suggested providing a list of anticipated adverse effects & a plan for detection/mitigation. IACUC unanimously agreed the re-submitted revised protocol be reviewed by DMR.

Follow-up meeting by sub-committee: On 10/31/2019 (9:30 – 10:30 am) the sub-committee met with the research associates from the (b)(6) lab. PI was not present. The associates presented historical data on the mortality rate and it was determined that the mortality followed a specific pattern based on whether it was an acute exposure study or a long-term study. In general, there was a lower incidence of mortality in acute studies (~15-20%) and a higher incidence of mortality (~40-70%) in the long-term recovery studies. The sub-committee suggested reporting these separately and reassessing animal requirement based on the type of study (short vs long) in an effort to reduce animal numbers.

CDC-Morgantown IACUC Policy P-009 USDA Pain Categories- discussed the examples of USDA Pain Category E- kept the antibody production- *in vivo* ascites methods in mice example and decided to omit language regarding food and water restrictions as this does not apply to research currently performed at NIOSH and if this type of research were performed it would be scrutinized during the protocol review process. The policy was unanimously approved with the recommended revision.

Topics for Discussion

Upcoming reviews for November meeting- amendment called for FCR during 48-hour amendment review; FCR of 3 new protocols

Next Meeting:

Next Meeting – Thursday, November 21, 2019 at 9:00 a.m. in (b)(7)(E)

Appendix 9.1- IACUC Protocol Form- Topaz New Protocol Form

Topaz New Protocol Form

Blank Form Report

Printed By (b)(6)

11/21/2019 7:52:38 AM

Report Comments

NIOSH-Morgantown Protocol Form (2.0)

Administrative Information

Reference Number System Generated 1.1

A unique Reference Number is used only by the TOPAZ database. It will not be used to identify the protocol

---

Title System Generated 1.2

Insert a descriptive protocol title. A good title includes the disease condition, if appropriate, the exposure or agent of interest, and the species. Limit the title to 150 characters

---

Protocol Type System Generated 1.3

Protocol Number System Generated 1.4

A unique protocol number will be assigned by the IACUC Administrator after approval

---

Principal Investigator System Generated 1.5

Select the Principal Investigator from the list. Do not leave blank; draft versions of the protocol can be saved only if a PI is selected

---

Branch or Department 1.6

Select the Branch or Department of the Principal Investigator

- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ Other

1.6.1

1.6.2

(b)(6)

1.6.3

1.6.4

1.6.5

1.6.6

1.6.7

1.6.8

Other Branch or Department 1.6.1

Enter the Branch or Department of the Principal Investigator

Emergency Contact List 1.7

Use the Add Row icon to enter the name(s) of the Principal Investigator and Protocol Associates authorized to make decisions regarding animal health and welfare. For each individual enter at least one daytime and one after-hours phone number (### ### ####). The first name in the emergency contact list must be the Principal Investigator.

Name	Daytime Phone Number	After-Hours Phone Number

Brief Description 1.8

Provide a brief 2-3 sentence summary of the study. Indicate the main independent variables, exposure methods, procedures, and general outcomes measures.

Accounts System Generated 1.9

Use the Add Accounts icon to enter the CAN(s) associated with the project. If you do not see the appropriate CAN listed, please contact the IACUC Administrator.

Funding Source for Purchase of Animals 1.10

Select the source of funding that will be used to purchase animals. (Who will own the animals?)

- ☐ [illegible]
- ☐ [illegible]

1.10.1

1.10.2

(b)(6)

Formal Administrative Agreements 1 11

Check YES if any of the animal work described is (or needs to be) covered by a formal agreement such as a Memorandum of Understanding (MOU), Materials Transfer Agreement (MTA), etc
Documentation of these agreement may be attached at the end of this document in the ATTACHMENTS section

☐ YES
☐ NO

1 11.1

Summary Description or Administrative Agreements 1 11 1 1

1 11.2

Sensitive or Proprietary Information 1 12

Does this research involve any sensitive or proprietary information that is privileged or confidential?

NOTE: It may not be necessary to describe sensitive or proprietary information in the protocol. Contact the IACUC Administrator for more information and guidance

☐ Yes
☐ No

1 12.1

1 12.2

Protocol Associates System Generated 1 13

Use the Add Associates icon to select all individuals that will be working on the protocol and their appropriate role(s). Select only individuals who will handle or perform procedures on animals.
Animal & Inhalation Facility Contractors and Some Histopathology Personnel should be added as a group. Scroll down to "Staff" in the personnel list to identify these groups as needed. The IACUC will monitor the training for these individuals. Contact the IACUC Administrator for a list of pre-qualified individuals if you have questions.

In the "Responsibilities" text field below each added Protocol Associate, list all specific procedures (e.g., specific non-surgical procedures (e.g. oropharyngeal aspiration, intrapentoneal injection, anesthesia by open-drop isoflurane anesthesia with ketamine cocktail), survival and non-survival surgeries (e.g. surgical placement of intra-abdominal transmitter, sub-cutaneous implant of osmotic pump, invasive plethysmography through tracheotomy, jugular/carotid cut down), euthanasia (e.g. barbiturate overdose and thoracotomy), etc.) that the individual will perform on animals. You must include sufficient detail that training and experience to perform the procedure can be assessed by the IACUC.

Co-Investigator: Co-Investigators have the same TOPAZ Elements access and editing privileges as the Primary Investigator.
Key Associate: Allows the individual to receive protocol related email messages but can only view the protocol in TOPAZ Elements.
Authorized to Order Animals: Allows the individual to place animal orders for the protocol in TOPAZ Elements.
Role Not Checked: Individual can only view the protocol in TOPAZ Elements.
NOTE: If an individual's name does not appear in the database, the individual may require training and authorization to work with animals. Contact the IACUC Administrator for more information and guidance on adding individuals to the database.

(b)(6)

Personnel Training and Experience 1.14

Check whether the Training and Experience Forms for the PI and every Protocol Associate are current and up-to-date. Please consult with the IACUC Administrator for guidance on training and experience requirements. Any individual, including the PI, who has not met or completed all IACUC training and experience requirements is not authorized to perform animal work under this protocol.

Note: Blank training and experience forms and all existing experience forms can be found at (b)(7)(E) It is the PI's responsibility to ensure that training forms for all protocol associates are kept up-to-date and saved in the shared folder.

☐ [illegible]

1.14.1

Animal Information

Species 2.1

Use the 'Add Species' icon to select the species of animal. Only one species per protocol is allowed.
Note: Some fields in this section may be hidden. Be sure to select the Expand All Items icon in the outline to see all relevant fields.

2.1.1

Mouse

Vendor and Strain/Stock/Breed System Generated 2.1.1.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts System Generated 2.1.1.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

Additional Animal Information 2.1.1.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon.

Sex	Age	Weight	Additional Features, Instructions, or Comments

2.1.2

Guinea Pig

Vendor and Strain/Stock/Breed System Generated 2.1.2.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

(b)(6)

Authorized Amounts — System Generated — 2.1.2.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B C, D or E Additional questions about how you determined the animal numbers will be asked in another section

Additional Animal Information — 2.1.2.3

Enter additional animal information that will be used for ordering/breeding/handling Additional rows can be added by clicking the Add Rows icon

Sex	Age	Weight	Additional Features, Instructions, or Comments

2.1.3

Mouse

Vendor and Strain/Stock/Breed — System Generated — 2.1.3.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested If your preferred vendor, strain stock, or breed is not listed please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts — System Generated — 2.1.3.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B C, D or E Additional questions about how you determined the animal numbers will be asked in another section

Additional Animal Information — 2.1.3.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon

Sex	Age	Weight	Additional Features, Instructions, or Comments

2.1.4

Rat

Vendor and Strain/Stock/Breed — System Generated — 2.1.4.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested If your preferred vendor, strain stock, or breed is not listed please contact the IACUC Administrator

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

(b)(6)

Authorized Amounts — By Item Generated — 2.1.4.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

Additional Animal Information — 2.1.4.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon.

Sex	Age	Weight	Additional Features, Instructions, or Comments

---

Breeding Colony — 2.2

Will you need to maintain a breeding colony? NOTE: IACUC protocol approval does not imply availability of resources (space and costs) for maintaining on site breeding colonies.

- ☐ [illegible]
- ☐ [illegible]

2.2.1

2.2.2

Justification for a Breeding Colony — 2.2.2.1

Justify the need to maintain a breeding colony in the text box below. See the IACUC Breeding Colony policy and complete a breeding roadmap and updates as requested by the holder of the approved IACUC breeding protocol.

Animal Identification — 2.3

Select the primary method that you will use for identifying animals. Cage cards will be placed by the Animal Facility.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

2.3.1

2.3.2

2.3.3

2.3.4

(b)(6)

2.3.5

Other Method of Animal Identification 2.3.5.1

List or briefly describe the other method for identifying animals. If the method involves an invasive procedure or has the potential to cause pain or distress, also describe this method in detail in the Procedures section

---

Animal Housing and Use Areas

Animal Housing Location(s) 3.1

Check the location(s) where animals will be housed. "Housing" refers to locations in which animals are kept for 12 hours or more. A separate question will ask about the location(s) where procedures will be performed on animals

☐ NIOSH Building

☐ Off-Site Location

3.1.1

Preferred NIOSH Housing Location(s) 3.1.1.1

Click the Add Rows icon to list the specific room locations where you wish to house animals. Also enter any additional features, equipment, or environmental requirements and the duration of housing

Building/Area/Room Number	Other requirements (e.g., fume hood, biosafety cab	Housing Durations

---

3.1.2

Off-Site Housing Location(s) 3.1.2.1

Use the Add Row icon to list every off-site animal housing location in the table below. A brief justification and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information

Institution/Department	Building/Room Number	Housing Durations	Brief Justification	AAALAC Accredited?	PHS Assurance Number

---

Individual(s) Responsible for Off-Site Housing 3.1.2.2

Enter the name of the individual responsible for the animals while they are housed off-site. Include emergency contact information (e.g., department, office, email, and day/evening phone numbers).

Animal Procedures Location(s) 3.2

Check the location(s) where procedures will be performed on live animals

☐ NIOSH Building

☐ Off-Site Location

3.2.1

(b)(6)

Location(s) of Animal Procedures and/or Drugs Intended for Animals 3.2.1.1

Click the Add Rows icon to list the specific NIOSH room locations where you wish to perform procedures on animals and where drugs intended for use in animals are kept. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) performed at each location.

NOTE: Include room locations where pharmaceutical drugs or other agents intended for use in animals will be stored even if no other procedures are performed there.

Room/Lab Number	List of Procedures	Are drugs or agents stored here?

3.2.2

Off-Site Procedures Location(s) 3.2.3.1

Use the Add Row icon to list every off-site location where procedures will be conducted. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) that will be performed on live animals at this off-site location. A brief justification for requiring off-site procedures and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information.

Institution/Department	Building/Room Number	List of	Brief Justification	AAALAC Accredited?	PHS Assurance Number

Individual(s) Responsible for Off-Site Procedures 3.2.3.2

Enter the name of the individual(s) responsible for conducting procedures on animals while they are used off-site. Include emergency contact information (e.g., department, office, email, and day and evening phone numbers).

Transport of Animals 3.3

Check how animals will be transported within the NIOSH facility or to and from other off-site locations. Check all that apply.

- ☐ [illegible]
- ☐ [illegible] (b)(4)
- ☐ [illegible]
- ☐ [illegible]

3.3.1

3.3.2

3.3.3

(b)(6)

Animal Transport by the PI, Laboratory Staff, or Other Personnel 3.3.3.1

Describe how animals will be transported within the NIOSH facility and name who will be responsible for transporting the animals. If available, attach copies of an SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

3.3.4

Animal Transport to or from an Off-Site Location 3.3.4.1

Describe how animals will be transported to or from an off-site location and name who will be responsible for transporting the animals. If available, attach copies of an SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

Study Objectives and Scientific Merit

Study Aims, Objectives, and Scientific Rationale 4.1

In the text field below, explain the specific aims, objectives, and scientific rationale of the study. Do not simply cut and paste from a scientific research proposal. The narrative should be written in language understandable to an educated layperson.

Role of Animals 4.2

In the text field below, briefly describe the role of animals in meeting the study objectives.
NOTE: You will also be asked to address alternatives to the use of animals in a separate section on the 3R's (refinement, reduction, replacement).

Potential Impact 4.3

In the text box below, explain the impact or benefit (e.g., health, safety, or the good of society) to be derived from the study.

Scientific Merit 4.4

How has the scientific merit of this study been evaluated? Check all that apply.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

4.4.1

(b)(6)

4.4.2

4.4.3

4.4.4

Other Method for Evaluating Scientific Merit or Additional Information 4.4.4.1

Briefly describe the other method(s) used to evaluate the scientific merit of the project

---

Duplication Assurance 4.5

Indicate whether the proposed project unnecessarily duplicates experiments conducted by you or other researchers.

☑ [illegible]

☐ [illegible]

4.5.1

Duplication Search Strategy and Results 4.5.1.1

Address the following two subparts
Describe the strategy you used to determine that the project does not duplicate previous experiments This is usually accomplished by including a description and results of a scientific literature review In such a description, include the databases searched relevant key search terms and number of hits obtained for each term and combination of terms
In a narrative provide your interpretation/evaluation of the search results
NOTE A table of the search strategy and results may be attached at the end of this document in the ATTACHMENT section Files must be closed before they can be attached

---

4.5.2

Duplication Justification 4.5.2.1

Cite and describe the previous study or studies being duplicated and provide a detailed scientific justification for the duplication

---

Study Design

(b)(6)

Study Description 5.1

In the text box below, provide a clear description of the experimental design and how animals are integrated with experimental procedures or conditions. Describe or show the assignment of animals to experimental groups or conditions, and specify the frequency, duration, timing, and/or sequence of procedures.
This description should allow the IACUC to understand the course of each animal through the study, from its arrival/acclimation to euthanasia. In this section, procedures should be named or described in only general terms; a separate section on Procedures will ask you for procedural details (e.g., routes of agents, anesthesia methods, potential adverse effects, etc.).

NOTE: Any documents used to illustrate what has been described may be added at the end of the document in the ATTACHMENT section. Files must be closed before they can be attached.

Animal Numbers, Justification, and Calculations 6.2

In the text box below, restate the total number of animals requested (from the section on Authorized Amounts), and show how the total number of animals is calculated, based on group sizes and study design (e.g., 8 animals per group x 5 experimental groups = 40 animals).
Also include a narrative that clearly explains the basis for determining that number using any or all of the following considerations:

The number of groups needed for a well-controlled experimental design.
The type of biological samples needed from animals, relative to the number of animals requested (e.g., quantity of sample that can be obtained from each animal).
The number of animals needed per group based on a statistical requirement, volume/number of biological samples needed, or both.
NOTE: Be as specific as possible. For example, if your study design includes multiple doses and time points or multiple control groups, be sure to provide a justification for these additional conditions. Any plan to repeat an experiment or include replicates usually is not approved in advance of need. If any repetitions or replicates are requested, provide the justification below.

Procedures

Use of Standard Operating Procedures (SOPs) 6.1

Will you be using NIOSH-approved SOPs that involve procedures conducted on live animals? Do not include SOPs that do not involve animal procedures.

- ☐ No
- ☐ Yes

6.1.1

6.1.2

(b)(6)

List of SOPs Involving Animal Procedures 6.1.2.1

For each SOP used, click the Add Row icon and fill in the requested information. Approved SOPs can be cited throughout this protocol form in lieu of describing the procedure details. A list of approved SOPs can be found at:

(b)(7)(E)

IMPORTANT! SOPs will be reviewed and approved by the IACUC every two (2) years. PIs will be notified in the event an SOP is revised. You must always use the most current version of the SOP. If you require any deviation from an approved SOP or latest version of an SOP, you must seek IACUC approval before using the procedure. An amendment may be required. See the IACUC Administrator for guidance.

SOP Number	SOP Title	SOP Version

Non-Surgical Procedures 6.2

Will non-surgical procedures be performed on live animals? Non-surgical procedures include whole body exposures, nose-only exposure, dermal application, injections, blood sampling, identification methods, etc., performed on live animals. NOTE: the description of common euthanasia methods are described in a later section.

- ☐ Yes
- ☐ No

6.2.1

6.2.2

Description of Non-Surgical Procedures 6.2.2.1

List and describe each non-surgical procedure or set of procedures (if performed at the same time point) that will be performed on live animals. Include the frequency and duration of the procedural treatments. Descriptions must include sufficient detail and a timeline to allow reviewers to understand what the animals will experience and the amount of pain or distress that might be anticipated.

Adverse Effects of Non-Surgical Procedures 6.2.3.1

Describe any adverse effects or clinical signs expected as a result of the non-surgical procedures. These might include pain, difficulty breathing, tumor development, weight loss, failure to thrive, and seizures, to name a few. In the text box below, organize/group the descriptions and clinical signs by different procedures, experiments, or set of studies, and list the humane endpoints, if applicable, relative to the experimental endpoints.

NOTE: Include possible effects or clinical signs that would be seen if inadequate/ineffective doses of anesthesia, analgesia, or sedation are given.

(b)(6)

Plan for Detection and Mitigation of Adverse Effects of Non-Surgical Procedures 6.2.1.3

Provide a plan for how animals will be monitored for pain/distress and the methods used to assess pain/distress. Plans should include frequency and duration of observations during critical periods, interventions (e.g., analgesia, thermal, or nutritional support) and humane endpoints relative to experimental endpoints. Indicate whether any specific metrics/tools will be used (e.g., pain scales, checklists, etc.).

NOTE: All possible adverse effects must be addressed in the plan.

Non-Survival Surgery 6.3

Will any invasive procedure or surgery be performed in which the animal is euthanized before recovery from anesthesia?

NOTE: Tissue collection or organ perfusion when the animal is unconscious following a fatal dose of euthanasia solution should be described in the separate section on Euthanasia.

☐ Yes

☐ No

6.3.1

6.3.2

Description of Non-Survival Surgery 6.3.2.1

Identify and describe in detail every non-survival surgical procedure. The description should include the surgical approach and the approximate duration of the procedures from anesthesia induction to euthanasia. If helpful, you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section. Be sure to include your anesthetic plan.

Note: The use of aseptic procedures and sterile instruments is recommended. The use of expired materials (e.g., drugs or supplies on live tissues) is prohibited unless specifically described in this section and approved by the IACUC.

Non-Survival Surgery and Responsible Personnel 6.3.2.2

Use the Add Rows icon to list every non-survival surgical procedure and then enter the name all individuals involved in the procedure(s).

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Intraoperative Monitoring during Non-Survival Surgery 6.3.2.3

Describe the steps planned to prevent or minimize pain or distress during the non-survival procedures. Include the plan for monitoring pain and distress, including the frequency and duration. Plans must be developed in consultation with and approved by the Attending Veterinarian.

(b)(6)

Survival Surgery 6.4

Will any animal undergo any survival surgery (i e be allowed to recover from anesthesia after surgery)?

☐ No

☐ Yes

6.4.1

6.4.2

Description of Survival Surgery 6.4.2.1

Describe every surgery including preoperative care, aseptic techniques, record keeping intra-operative monitoring and support post-operative care name and qualifications of the surgeon(s). If you purchase surgically manipulated animal models you must have approval from the Attending Veterinarian and may require an MOU and must include post-operative care plan in the section below Include here your anesthetic plan including induction, maintenance and analgesia Your plan for analgesia should extend through anesthetic recovery and return to housing
If helpful, you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section Be sure to include your anesthetic plan

Survival Surgery and Responsible Personnel 6.4.2.2

Use the Add Rows icon to list every survival surgical procedure and then enter the name all individuals involved in the procedure(s):

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Post-operative Plan and Support following Survival Surgery 6.4.2.3

Describe the steps planned to prevent or minimize pain or distress during and after survival surgery Include the plan for monitoring pain and distress, including the frequency and duration Wound care must be described, and plans should be developed in consultation with and approved by the Attending Veterinarian

Experimental Agents Drugs, and Substances Used during Procedures 6.5

Use the Add Rows icon to enter every agent, drug or substance, including euthanasia agents that will be used during the procedures described above or during any time of study period For any euthanasia agent such as pentobarbital or isoflurane, ensure that the dose, route, and schedule, etc match what is selected or described below in the section on Euthanasia. If necessary scroll across the page to see all columns
Note Use the blue "?" button to the right for appropriate 'Agent Type' categories Contact IACUC Administrator if additional terms need to be added
Note Please also indicate in the previous sections, where appropriate, how and when these agents drugs or substances are used in conjunction with the various non-surgical or surgical procedures

Agent Type (see help)	Name of Agent	Dose or Amount	Route	Frequency or Schedule	Comments

(b)(6)

Controlled (CII or CIII) Substances 6.6

Are any of drugs entered above a controlled (CII or CIII) substance? Contact the Attending Veterinarian for guidance NOTE: Euthanasia solution containing pentobarbital is a controlled substance and must be listed here

- ☐ No
- ☐ Yes

6.6.1

6.6.2

Controlled Substances 6.6.2.1

For any controlled substances identified above, click the Add Rows icon, select the substance from the list, and then provide the information requested in the other columns. All persons listed should be named as Protocol Associates above and have training regarding the Controlled Substances Act and local requirements. Contact the Attending Veterinarian for assistance.

Name of Controlled Substance(s)	Name of Person(s) with Access	Name of Person(s) that will be dosing	Room Number Where Controlled Drugs will be Stored	Person(s) Responsible for Recordkeeping

Antemortem Sample or Tissue Collection 6.7

Will any biologic or tissue samples be collected from animals before they are euthanized?

- ☐ No
- ☐ Yes

6.7.1

6.7.2

Antemortem Sample or Tissue Collection 6.7.2.1

Use the Add Rows icon to enter the type of tissues or samples to be collected, the amount, volume, or size of sample or tissue collected, the site (body location), and frequency or schedule of sample or tissue collection. If any drugs will be used to alleviate potential pain or distress, be sure to include them in the section on Analgesia, Anesthesia, and Sedation

Sample or Tissue Type	Amount, Volume, Size	Site	Frequency or Schedule	Other Comments

Genetically Modified (GM) Rodent Use in Experiments 6.8

Check all that apply

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

6.8.1

(b)(6)

6.8.2

6.8.3

6.8.4

6.8.5

Anticipated Adverse Effects in GM Animals 6.9

As a result of the genetic modification, do you anticipate these animals will experience adverse effects above and beyond what is expected in wild type controls?
NOTE: Any unanticipated or unexpected problems or adverse effects that are not explicitly described in the protocol may be subject to reporting requirements to institutional and regulatory officials and must be reported to the IACUC and AV promptly.

☐ [illegible]
☐ [illegible]

6.9.1

6.9.2

Description of Possible or Expected Adverse Effects in GM Animals 6.9.2.1

Describe the possible or expected adverse effects in two parts: (1) Describe all possible or expected adverse effects including any veterinary/housing problems that are possible or expected; and (2) Describe the humane endpoints or criteria for euthanasia if applicable. Lastly, provide any plans for additional supportive care to improve the animal condition that will not interfere with experimental endpoints.

Special Concerns for Animal Use

Deviation from Social Housing (ref: IACUC policy) 7.1

Will animals need to be single housed?
NOTE: Single housing may be approved only with a scientific justification or a veterinary-approved behavioral or health reason. The duration of single housing must be minimized, and animals should not be held for extended periods in social isolation awaiting the start of the study. Click the Help icon for more information or contact the IACUC Administrator for guidance.

☐ [illegible]
☐ [illegible]

7.1.1

7.1.2

(b)(6)

Justification for Single Housing 7.1.2.1

Consistent with current animal welfare regulations, policies and AAALAC recommendations, the health and welfare benefits of social housing are enough to consider it the default condition. Therefore, an adequate justification for single housing must include empirical evidence from the scientific literature or experience that supports the conclusion that group housing will negatively affect the study objectives or results. An adequate justification must also weigh the potential adverse effects of single housing. Behavioral and health concerns (e.g., aggression in C57BL/6 male mice) and some restrictive grouping/blocking designs may also justify single housing, but these scenarios also require detailed explanations.

NOTE: For more information, see the IACUC Policy on Social Housing, which can be found here.

Acclimation of Animals after Arrival 7.2

Will animals be acclimated to the facility for at least 5 days after arrival and before procedures are performed?

☐ Yes (default)

☐ No

7.2.1

7.2.2

Alternative Acclimation Procedure 7.2.2.1

Describe and justify the alternative acclimation procedure.

Withholding of Enrichment - Complete this section for all new submissions after September 1, 2017 7.3

See the species-specific enrichment choices below. Conditional items may be introduced with AV and PI approval to address behavioral or health issues.

For Mice:

Default - nesting material (Depending on the strain, crinkle paper may be used alone or in combination with nesting squares.)

Conditional - structures such as shelves to reduce unwanted behaviors such as plugging water bottles, wheels or food treats

For Rats

Default - plastic tubes/tunnels and chew/gnawing substrate (Nylabone or certified wood blocks).

Conditional - crinkle nesting material with or without nesting squares, kiln dried Aspen shavings instead of wood chip for burrowing or foodtreats.

For Guinea Pigs

Default - shelter

Conditional - food treats

☐ [illegible]

☐ [illegible]

☐ [illegible]

☐ [illegible]

7.3.1

(b)(6)

7.3.2

7.3.3

7.3.4

Enrichment Restrictions and Plans 7.4

In the text box below, (1) provide scientific justification applicable to your research for providing no enrichment (2) identify which groups of animals this restriction applies and (3) the plan for how the period of restriction will be minimized

---

Limitations of Enrichment 7.6

Use this section to describe any limitations you have regarding enrichments listed on the species-specific default list above. You must provide a scientific rationale that is applicable to your work

---

Regrouping after Acclimation 7.6

Will a regrouping of socially housed animals (change in social group) be required after acclimation or at any time during the study period?

☐ No, [illegible]

☐ Yes

7.6.1

7.6.2

Justification for Regrouping after Acclimation 7.6.2.1

Provide a description and justification for regrouping. Explain why it is necessary and why it will not adversely affect your study objectives

NOTE: Regrouping animals after acclimation induces significant stress and may lead to aggression/fighting. Methods are available to mitigate this stress. Please contact the Attending Veterinarian for guidance.

Nutritional Restrictions or Additives 7.7

Will the animals receive a non-standard food or water regimen?

☐ No, [illegible]

☐ Yes

7.7.1

7.7.2

(b)(6)

Justification for a Non-Standard Food or Water Regimen 7.7.2.1

Address the following three subparts:

1. describe with details the non-standard food or water regimen, including time of day and duration of withdrawal
2. provide a detailed justification
3. describe specific actions to monitor animals for adverse health effects (such as food/water refusal and/or weight loss)

NOTE: Any additives to food or water must be documented in the Study Design and Safety Sections

---

Non-Standard Environment (ref. Satellite Housing policy) 7.8

Will animals be exposed to any environment or condition that is different from the standard home cage environment? Common non-standard environments in our facility include whole body inhalation exposure chambers, metabolic cages, plethysmographs, environmental chambers, operant chambers, etc.

☐ No, Default

☐ Yes

7.8.1

7.8.2

Justification of Non-Standard Environment(s) 7.8.2.1

Address the following subparts:

1. describe the non-standard environment
2. provide a detailed justification
3. describe how animals will be acclimated to the non-standard environment
4. describe specific steps taken to monitor animals for adverse health effects
5. for Satellite Facilities, provide justification for housing animals outside of the animal facility

NOTE: For specialized chambers or animal holding devices, specify details of the environment including its dimensions, air handling and temperature control, duration and frequency of exposures, etc. If helpful, diagrams of the non-standard environment may be attached using the Add Attachments/Links icon.

Restraint: Prolonged or Non-Routine 7.9

Will animals require prolonged (i.e., greater than 5 minutes) physical restraint or be confined to a device which prevents normal movement (e.g., nose-only inhalation chambers)?

NOTE: Momentary manual restraint methods (e.g., handling, Decapicone bags, commercial rodent restrainers), commonly used for drug injections, intranasal instillations, or similar procedures are not considered prolonged restraint. Acclimation to devices and gentle handling prior to the restraint are required to reduce stress in animals. Restraint duration should be limited to the minimum that is scientifically necessary. Any observed trauma or distress as a result of the restraint must be promptly reported to the Attending Veterinarian.

☐ No

☐ Yes

(b)(6)

7.9.1

7.9.2

Justification for Prolonged or Non-Routine Restraint 7.9.2.1

Address the following four subparts

1 describe the restraint device or method
2 provide a detailed justification
3 describe how animals will be acclimated to the restraint
4 describe specific steps taken to monitor animals for adverse health effects

NOTE If helpful diagrams of the restraint device may be attached in the ATTACHMENT section at the end of this document

---

Multiple Survival Surgeries 7.10

Will multiple survival surgeries be performed on the same animal?

☐ Yes
☐ No

7.10.1

7.10.2

Use of Pharmaceutical Grade Drugs or Agents 7.11

Are all drugs or agents to be used in live animals Pharmaceutical Grade? If there are any exceptions (e.g., if you must use a reagent grade agent or a USP formulation that is not pharmaceutical grade), check No

☐ Yes
☐ No

7.11.1

(b)(6)

Description and Justification for Non-Pharmaceutical Grade Drugs or Agents 7.11.1.1

By default, drugs or agent that are used but not included in this table, must be Pharmaceutical Grade. For each non-pharmaceutical grade drug or agent used, click the Add Rows icon and then provide the requested information under each column:

1. name of the drug or agent
2. brief justification for its use (e.g., "pharmaceutical grade is not available")
3. information about the source (e.g., vendor)
4. preparation procedures (e.g., reconstitution, dilution, mixing, final concentrations, aseptic handling, stability, storage)
5. quality control (purity, grade, sterility, pyrogenicity, pH, or other property)

NOTE: Not all USP formulations are pharmaceutical grade; reagents are not pharmaceutical grade compounds. When mixing reagents to be used in live animals, you must describe preparation procedures and, at a minimum, provide information on pH and how you will ensure sterility.

Name of Drug or Agent	Justification	Source	Preperation Procedures	Purity, grade, sterility, pH, pyrogenicity, etc.

7.11.2

USDA Category E Animals (Unrelieved pain, distress or physiologic impairment) 7.12

In the earlier section on Animal Information, were any animals assigned to USDA Category E (Category III)?

- ☐ No
- ☐ Yes

7.12.1

7.12.2

USDA Category E (Category III) Scientific Justification and Humane Endpoints 7.12.2.1

USDA Category E procedures require additional information. Address each of the following three subparts:

1. Provide a detailed scientific justification for withholding anesthetics, analgesics, or sedatives.
2. Describe the steps taken to monitor the health status of the animals.
3. List the humane endpoints (i.e., signs or symptoms that will prompt euthanasia or removal from the study).

The Three Rs

(b)(6)

Refinement, Replacement, and Reduction 8.1

Investigators must show how they have considered alternatives methods and procedures that address animal welfare concerns. A consideration of alternatives is usually addressed by responding to "the Three Rs" (refinement replacement and reduction)
Refinement - decreasing pain or distress by modifying the husbandry or experimental procedures
Replacement - using methods that avoid or replace the use of animals by substitution with non-animal systems or less sentient animals
Reduction - using strategies that result in fewer animals being used or maximizing the information obtained from the number of animals being used
For help with these concepts and requirements go to http://www.nal.usda.gov/alternatives or http://vetmed.duhs.duke.edu/GuidelinesforAlternativeSearching.html or http://altweb.jhsph.edu/

Refinement 8.2

For each procedure listed in Procedures section that causes pain or distress or has the potential to cause pain or distress describe the steps taken to minimize pain or distress or reduce the adverse effects of the procedures

---

Refinement: Supporting Evidence 8.3

Provide evidence of your consideration of refinements to procedures that may cause pain or distress Address the following three subparts

1 List the databases searched (or other sources consulted), the years covered the key words or search strategy used, and the date of the search. At minimum, the key words should include comprehensive search terms (or MESH terms) for each procedure that has the potential to cause pain or distress and for alternative procedures
2 Describe the search results including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results.
3 List and describe any additional methods (other than a literature search) that were used in considering refinements (e g experts, standards, regulations, conference proceedings etc)

NOTE You may attach a table of the search results in the ATTACHMENT section at the end of this document

---

Replacement 8.4

Provide the rationale for using animals rather than non-animal alternatives and provide a rationale for the chosen species.

(b)(6)

Replacement: Supporting Evidence 3.6

Provide evidence of your consideration of replacements for animals. Address the following three suoparts

:1 List the databases searched (or other sources consulted), the years covered, the key words or search strategy used and the date of the search. At minimum, key words should include comprehensive search terms (or MESH terms) for non-animal models and systems (e.g., in vitro, cell culture, computer modeling, simulations, etc.)
:2 Describe the search results, including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results
:3 List and descnbe any additional methods (other than a literature search) that were used in considenng replacements

NOTE: You may attach a table of the search results in the ATTACHMENT section at the end of the document. Files must be closed before they can be attached

---

Reduction of Animal Numbers 3.8

Descnbe the steps taken to reduce, minimize, and/or optimize the number animals being used. Include any statistical plan used to achieve reduction/optimization

NOTE: Reduction refers to efforts that minimize the numbers of animals used. Reduction is addressed by discussing the minimum numbers of animals needed for yielding sufficient samples, the appropnate number animals to achieve statistically significant results, or an experimental design that minimizes the required numoer of animals.

Euthanasia and Disposition

Euthanasia Methodisn 9.1

From the list of IACUC-approved methods below, select how the animals will be euthanized as part of the experimental protocol. If multiple methods will be used across different groups of animals or different experiments, descnbe this in the section on Procedures. Any euthanasia method that deviates from the following specific methods requires the selection of Other and a detailed descnption.
NOTE: More information about acceptaole methods can be found in the NIOSH IACUC policy on Euthanasia and the AVMA Guidelines on Euthanasia.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

9.1.1

9.1.2

(b)(6)

9.1.3

9.1.4

Plan Description for Meeting the Euthanasia Method Conditions 9141

The AVMA Guidelines for Euthanasia of Animals considers the physical methods described above as "Acceptable with Conditions." As the PI you must provide details on how you will meet those conditions for the method(s) you have chosen. In addition, conscious decapitation requires a scientific justification that explains why this method is required over another accepted method that does not have additional conditions. Please address this in the text box below. See the ACUC Euthanasia policy and SOP for help.

9.1.5

9.1.6

9.1.7

9.1.8

9.1.9

Other Euthanasia Method 9181

Describe the method of euthanasia not listed specifically above. In addition, for any euthanasia method not described as "Acceptable" or "Acceptable with Conditions" in the AVMA Guidelines on Euthanasia, provide a scientific justification. For methods described as "Acceptable with Conditions," describe how the conditions will be met and how training to meet those conditions will be obtained. Please seek a veterinary consult for additional information or guidance.

(b)(6)

Disposition of Samples or Tissues Collected Post-Mortem 9.2

Check all that apply. The purpose of this section is to assist with the clearance of publications, posters, and scientific presentations per NIOSH/HELD internal policy, as well as journal requirements for a statement attesting that "animal work was covered by an IACUC approved protocol."
If your intent prior to euthanasia is to share or collect tissue for purposes not within the scope and objectives of this protocol, then you must declare that intent. The IACUC fully supports the concept of tissue sharing and reduction of animal use; however, if there is intent to bank tissues for other uses even though they may be directly or indirectly related to this protocol, then this intent must be documented. If your intent changes during the protocol period, please notify the IACUC to request a change in your answer to this question. (This change can be done administratively.)

Note also:
1. All samples/tissues collected must be identified with a CDC identifier
2. All samples must be transported consistent with state and federal regulations
3. All samples that are considered biohazardous may not leave the building without formal approval of the Health and Safety Office
4. Even with a declaration of intent to share samples or tissue, animals approved under this protocol must not be euthanized for the sole purpose of providing samples or tissues outside of the study objectives without prior IACUC approval

☐ [illegible]

☐ [illegible]

☐ [illegible]

9.2.1

9.2.2

9.2.3

Safety and Health

Hazardous Agents System Generated 10.1

Using the Add Hazardous Agents icon, select from the list ALL agents (hazardous or not) administered to animals as part of your experiments. The agents listed must match the list of agents provided in the separate hazardous agents attachment described below. If a specific agent does not appear in the list, contact the IACUC Administrator.

Additional Precautions for Animal Care Personnel 10.2

Describe any additional engineering controls, specific instructions for animal husbandry, or specific work practices for animal care personnel.

Additional Precautions for Laboratory Personnel 10.3

Describe any additional engineering controls, specific work practices, or other specific instructions for handling hazardous agents or conducting procedures.

(b)(6)

Bedding Disposal Procedures 10.4

Indicate whether routine or standard bedding disposal procedures are acceptable

☐ [illegible]

☐ [illegible]

10.4.1

10.4.2

Other Bedding Disposal Procedures 10.4.2.1

Describe the non-routine or special bedding disposal procedures

---

Special Carcass Removal Procedures 10.5

Indicate whether routine or standard carcass removal procedures are acceptable

☐ [illegible]

☐ [illegible]

10.5.1

10.5.2

Description of Special Carcass Removal Procedures 10.5.2.1

Describe the non-routine or special precautions for carcass removal

---

Special Decontamination Procedures 10.6

Indicate whether routine or standard decontamination procedures are acceptable

☐ [illegible]

☐ [illegible]

10.6.1

10.6.2

Description of Special Decontamination Procedures 10.6.2.1

Describe non-routine or special precautions for decontamination procedures

---

Investigator Assurances & Compliance Agreement

(b)(6)

Principal Investigator Assurances and Compliance Agreement 11.1

I will conduct this study in accordance with all applicable rules and regulations as described in the PHS Policy on Humane Care and Use of Laboratory Animals the Federal Animal Welfare Act and Regulations. the Guide for the Care and Use of Laboratory Animals. and CDC/NIOSH Morgantown internal policies (Click the Help icon for links to these resources.)

I will obtain approval from the IACUC before initiating any change in the study objectives. design. or procedures I understand that work performed without IACUC approval cannot be published with certification of IACUC approval and may result in federally-required reporting of noncompliance

I have determined that the research proposed is not unnecessarily duplicative

I have reviewed the pertinent scientific literature and the sources and/or databases noted in this proposal and found no scientifically acceptable alternatives to any procedures that may cause pain or distress

I confirm that all individuals working on this protocol are enrolled in NIOSH's Occupational Health and Safety Program

I authorize individuals listed on this protocol to conduct procedures involving animals. and I accept responsibility for their oversight in the conduct of this proposal. I certify that these same individuals will read. understand and follow the procedures in the final approved version of this protocol and any future amendments

I confirm that all individuals listed on this protocol have obtained authorization to work with animals in this facilrty by the Animal Facilities Director or will be required to do so before work with animals begins Further. I certify that these individuals are properly trained or will receive such training prior to work with animals in all areas relevant to their assigned work with animals (e g biology, handling. and care of the species used aseptic surgical methods and techniques. the concept of 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 and procedures for reporting animal welfare concerns.)

I certify that all individuals listed on this protocol will complete any additional training continuing education or refresher training as assigned by the IACUC

I understand that I am responsible for assuring that my laboratory is in compliance with all federal state and local environmental laws and regulations I accept the responsibilry to assure that my laboratory is operated in a safe manner and that all staff and students are informed of potential risks wear appropriate personal protective equipment. and are adequately trained I will assure that all personnel working on this protocol receive orientation to laboratory safety instructions SDS files. and laboratory emergency procedures. I am responsible for adhering to NIOSH safety policies and procedures for handling hazardous materials and for addressing accidental spills and personnel contamination I will report any significant problems. and research-related accidents and illnesses to the Safety Office and will complete the required forms in the event of an incident

For animals under this protocol. I accept that in the case of necessary veterinary treatment. the Attending Veterinarian is authorized to provide treatment required to sustain life or if necessary, provide humane euthanasia to prevent unapproved pain and/or distress. I understand that the animal facility staff will contact me as soon as possible using the emergency contact information that I provide in this protocol but I understand that such contact may not always be possible prior to treatment or performing euthanasia

I will notify the IACUC of unanticipated adverse outcomes Unanticipated outcomes are generally defined as negative impacts to animal welfare or well-being that are not explicitly described in the protocol and approved by the IACUC These include unanticipated outcomes associated with disease. injury. or effects that are under investigation

I accept that veterinary consultation must occur when pain or distress is beyond the level the IACUC approved in the protocol. or when animal facility staff are unable to provide interventional control (i e immediate euthanasia) I will notify the Attending Veterinarian when unanticipated pain or distress unexpected morbidity or unanticipated mortality occurs This applies to animals housed/used at NIOSH and animals funded by NIOSH and housed/used at collaborating institutions.

☐ [illegible]

11.1.1

(b)(6)

ATTACHMENT Section 11.1

Use this section to attach any documents used to illustrate what you have written in the sections above. Keep in mind that attachments are not printed out with the protocol and that the information provided in the protocol must stand on its own.
The IACUC prefers files in pdf (images). WORD and Excel files should be saved as pdf before attachment.
Attach the file using the paperclip icon at the right.
For each file attached, complete the description below.

Date Document Added	File Name	File Content/Description	Protocol Sections

For IACUC Administration Use Only

IACUC Admin Section 12.1

This section will be used for the IA to add correspondence or other administrative documents.

☐ [illegible]
☐ N.

12.1.1

12.1.2

Comments 12.2

July 2020 update to Appendix 9.1: IACUC Protocol Form

Added Section 7.13 Management of Ulcerative Dermatitis and Exercising Humane Endpoints

- Chronic, ulcerative dermatitis is a common problem, particularly in aging mice on a C57 background. It often responds poorly to treatment, leaving the animal in a constant state of distress and sometimes pain.

Will you fully comply with AF Working Standard V-03, Mouse Ulcerative Dermatitis and Humane Endpoints?

__ Yes, I will fully comply
__ *No, I am unable to comply and wish to be exempted from this Standard
__ N/A (for rats and mice that won't exceed six months of age)

* 7.13.2.1 Reasons for Exemption
Please state the reasons you wish to be exempted from this Standard

Appendix 9.2: IACUC Protocol Form
Topaz Amendment to Approved Protocol Form

Blank Form Report

Printed By: (b)(6)

Report Comments

NIOSH Morgantown Amendment Form v2.0

Amendment Summary

Requested Changes 1.1

Select the protocol change(s) being requested. Check all that apply.
Click the Help icon for more information and guidance on what constitutes a significant protocol change that requires ACUC approval.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ Other

1.1.1

1.1.2

1.1.3

1.1.4

1.1.5

1.1.6

1.1.7

1.1.8

1.1.9

(b)(6)

Summary of Changes and Justification 1.2

Briefly describe in summary form all changes to the protocol in the text box below and briefly explain why each change is needed. Then revise all relevant sections of the protocol.

Note: Provide only a summary of the changes in the text box below. All changes must also be addressed in detail in the relevant sections of the protocol form. Click the Help icon for more information and guidance on requesting changes to an approved protocol.

Administrative Information

Reference Number System Generated 2.1

A unique Reference Number is used only by the TOPAZ database. It will not be used to identify the protocol.

Title System Generated 2.2

Insert a descriptive protocol title. A good title includes the disease condition, if appropriate, the exposure or agent of interest, and the species. Limit the title to 150 characters.

Protocol Type System Generated 2.3

Protocol Number System Generated 2.4

A unique protocol number will be assigned by the IACUC Administrator after approval.

Principal Investigator System Generated 2.6

Select the Principal Investigator from the list. Do not leave blank; draft versions of the protocol can be saved only if a PI is selected.

(b)(6)

Branch or Department 2.6

Select the Branch or Department of the Principal Investigator

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

2.6.1

2.6.2

2.6.3

2.6.4

2.6.5

2.6.6

2.6.7

2.6.8

Other Branch or Department 2.6.1

Enter the Branch or Department of the Principal Investigator

Emergency Contact List 2.7

Use the Add Row icon to enter the name(s) of the Principal Investigator and Protocol Associates authorized to make decisions regarding animal health and welfare. For each individual enter at least one daytime and one after-hours phone number (###-###-####). The first name in the emergency contact list must be the Principal Investigator

Name	Daytime Phone Number	After-Hours Phone Number

Brief Description 2.8

Provide a brief 2-3 sentence summary of the study. Indicate the main independent variables, exposure methods, procedures, and general outcomes measures.

(b)(6)

Accounts System Generated 2.9

Use the Add Accounts icon to enter the CAN(s) associated with the project If you do not see the appropriate CAN listed please contact the IACUC Administrator

Funding Source for Purchase of Animals 2.10

Select the source of funding that will be used to purchase animals (Who will own the animals?)

- [ ] NIH
- [ ] [illegible]

2.10.1

2.10.2

Formal Administrative Agreements 2.11

Check YES if any of the animal work described is (or needs to be) covered by a formal agreement such as a Memorandum of Understanding (MOU), Materials Transfer Agreement (MTA), etc
Documentation of these agreement may be attached at the end of this document in the ATTACHMENTS section

- [ ] YES
- [ ] NO

2.11.1

Summary Description of Administrative Agreements 2.11.1.1

2.11.2

Sensitive or Proprietary Information 2.12

Does this research involve any sensitive or proprietary information that is privileged or confidential?

NOTE It may not be necessary to describe sensitive or proprietary information in the protocol Contact the IACUC Administrator for more information and guidance.

- [ ] NO
- [ ] YES

2.12.1

2.12.2

(b)(6)

Protocol Associates — System Generated — 2.13

Use the Add Associates icon to select all individuals that will be working on the protocol and their appropnate role(s).
Select only individuals who will handle or perform procedures on animals.
Animal & Inhalation Facility Contractors and Some Histopathology Personnel should be added as a group. Scroll down to Staff in the personnel list to identify these groups as needed. The IACUC will monitor the training for these individuals.
Contact the IACUC Administrator for a list of pre-qualified individuals if you have questions.

In the "Responsibilities" text field below each added Protocol Associate, list all specific procedures (e.g., specific non-surgical procedures (e.g., oropharyngeal aspiration, intraperitoneal injection), anesthesia by open-drop isoflurane, anesthesia with ketamine cocktail), survival and non-survival surgeries (e.g., surgical placement of intra-abdominal transmitter, sub-cutaneous implant of osmotic pump, invasive plethysmography through tracheotomy, jugular/carotid cut down), euthanasia (e.g., barbiturate overdose and thoracotomy), etc.) that the individual will perform on animals. You must include sufficient detail that training and experience to perform the procedure can be assessed by the IACUC.

Co-Investigator: Co-Investigators have the same TOPAZ Elements access and editing privileges as the Primary Investigator.
Key Associate: Allows the individual to receive protocol related email messages but can only view the protocol in TOPAZ Elements.
Authorized to Order Animals: Allows the individual to place animal orders for the protocol in TOPAZ Elements.
Role Not Checked: Individual can only view the protocol in (b)(7)(E).
NOTE: If an individual's name does not appear in the database, the individual may require training and authorization to work with animals. Contact the IACUC Administrator for more information and guidance on adding individuals to the database.

Personnel Training and Experience — 2.14

Check whether the Training and Experience Forms for the PI and every Protocol Associate are current and up-to-date.
Please consult with the IACUC Administrator for guidance on training and experience requirements. Any individual, including the PI, who has not met or completed all IACUC training and experience requirements is not authorized to perform animal work under this protocol.
Note: Blank training and experience forms and all existing experience forms can be found at (b)(7)(E). It is the PI's responsibility to ensure that training forms for all protocol associates are kept up-to-date and saved in the shared folder.
☐ [illegible]

2.14.1

Animal Information

Species — 3.1

Use the "Add Species" icon to select the species of animal. Only one species per protocol is allowed.
Note: Some fields in this section may be hidden. Be sure to select the Expand All Items icon in the outline to see all relevant fields.

3.1.1

[illegible]

Vendor and Strain/Stock/Breed — System Generated — 3.1.1.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

(b)(6)

Authorized Amounts — System Generated — 3.1.1.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

Additional Animal Information — 3.1.1.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon.

Sex	Age	Weight	Additional Features, Instructions, or Comments

3.1.2

Rabbit

Vendor and Strain/Stock/Breed — System Generated — 3.1.2.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts — System Generated — 3.1.2.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

Additional Animal Information — 3.1.2.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon.

Sex	Age	Weight	Additional Features, Instructions, or Comments

3.1.3

Swine

Vendor and Strain/Stock/Breed — System Generated — 3.1.3.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

(b)(6)

Authorized Amounts — System Generated — 3.1.3.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropnate USDA pain category B C, D or E Additional questions about how you determined the animal numbers will be asked in another section

Additional Animal Information — 3.1.3.3

Enter additional animal information that will be used for ordering/breeding/handling Additional rows can be added by clicking the Add Rowsicon

Sex	Age	Weight	Additional Features, Instructions, or Comments

3.1.4

Rat

Vendor and Strain/Stock/Breed — System Generated — 3.1.4.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested If your preferred vendor, strain stock, or breed is not listed please contact the IACUC Administrator

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts — System Generated — 3.1.4.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B C, D or E. Additional questions about how you determined the animal numbers will be asked in another section

Additional Animal Information — 3.1.4.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rowsicon

Sex	Age	Weight	Additional Features, Instructions, or Comments

Breeding Colony — 3.2

Will you need to maintain a breeding colony? NOTE IACUC protocol approval does not imply availability of resources (space and costs) for maintaining on site breeding colonies

- ☐ No
- ☐ Yes

3.2.1

3.2.2

(b)(6)

Justification for a Breeding Colony 3.2.2.1

Justify the need to maintain a breeding colony in the text box below. See the IACUC Breeding Colony policy and complete a breeding roadmap and updates as requested by the holder of the approved IACUC breeding protocol

---

Animal Identification 3.3

Select the primary method that you will use for identifying animals. Cage cards will be placed by the Animal Facility

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

3.3.1

3.3.2

3.3.3

3.3.4

3.3.5

Other Method of Animal Identification 3.3.6.1

List or briefly describe the other method for identifying animals. If the method involves an invasive procedure or has the potential to cause pain or distress, also describe this method in detail in the Procedures section

---

Animal Housing and Use Areas

Animal Housing Location(s) 4.1

Check the location(s) where animals will be housed. "Housing" refers to locations in which animals are kept for 12 hours or more. A separate question will ask about the location(s) where procedures will be performed on animals

- ☐ [illegible]
- ☐ [illegible]

4.1.1

Preferred NIOSH Housing Location(s) 4.1.1.1

Click the Add Rows icon to list the specific room locations where you wish to house animals. Also enter any additional features, equipment, or environmental requirements and the duration of housing

Building/Area/Room Number	Other requirements (e.g., fume hood, biosafety cab)	Housing Durations

---

(b)(6)

4.1.2

Off-Site Housing Location(s) 4.1.2.1

Use the Add Row icon to list every off-site animal housing location in the table below. A brief justification and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information.

Institution/Department	Building/Room Number	Housing Durations	Brief Justification	AAALAC Accredited?	PHS Assurance Number

Individual(s) Responsible for Off-Site Housing 4.1.2.2

Enter the name of the individual responsible for the animals while they are housed off-site. Include emergency contact information (e.g., department, office, email, and day/evening phone numbers).

Animal Procedures Location(s) 4.2

Check the location(s) where procedures will be performed on live animals.

- ☐ [illegible]
- ☐ [illegible]

4.2.1

Location(s) of Animal Procedures and/or Drugs Intended for Animals 4.2.1.1

Click the Add Rows icon to list the specific NIOSH room locations where you wish to perform procedures on animals and where drugs intended for use in animals are kept. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) performed at each location.

NOTE: Include room locations where pharmaceutical drugs or other agents intended for use in animals will be stored, even if no other procedures are performed there.

Room/Lab Number	List of Procedures	Are drugs or agents stored here?

4.2.2

Off-Site Procedures Location(s) 4.2.2.1

Use the Add Row icon to list every off-site location where procedures will be conducted. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) that will be performed on live animals at this off-site location. A brief justification for requiring off-site procedures and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information.

Institution/Department	Building/Room Number	List of	Brief Justification	AAALAC Accredited?	PHS Assurance Number

(b)(6)

Individual(s) Responsible for Off-Site Procedures 4.2.2.2

Enter the name of the individual(s) responsible for conducting procedures on animals while they are used off-site. Include emergency contact information (e.g., department, office, email, and day and evening phone numbers).

Transport of Animals 4.3

Check how animals will be transported within the NIOSH facility or to and from other off-site locations. Check all that apply.

- ☐ [illegible]
- ☐ [illegible] (b)(4)
- ☐ [illegible]
- ☐ [illegible]

4.3.1

4.3.2

4.3.3

Animal Transport by the PI, Laboratory Staff, or Other Personnel 4.3.3.1

Describe how animals will be transported within the NIOSH facility, and name who will be responsible for transporting the animals. If available, attach copies of an SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

4.3.4

Animal Transport to or from an Off-Site Location 4.3.4.1

Describe how animals will be transported to or from an off-site location, and name who will be responsible for transporting the animals. If available, attach copies of an SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

Study Objectives and Scientific Merit

Study Aims, Objectives, and Scientific Rationale 6.1

In the text field below, explain the specific aims, objectives, and scientific rationale of the study. Do not simply cut and paste from a scientific research proposal. The narrative should be written in language understandable to an educated layperson.

(b)(6)

Role of Animals 5.2

In the text field oelow, briefly describe the role of animals in meeting the study objectives
NOTE You will also be asked to address alternatives to the use of animals in a separate section on the 3R's (refiinement reduction replacement).

Potential Impact 5.3

In the text box oelow explain the impact or benefit (e g., health, safety, or the good of society) to be derived from the study

---

Scientific Merit 5.4

How has the scientific merit of this study been evaluated? Check all that apply

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

5.4.1

5.4.2

5.4.3

5.4.4

Other Method for Evaluating Scientific Merit or Additional Information 5.4.4.1

Bnefly describe the other methodis) used to evaluate the scientific merit of the project

---

Duplication Assurance 5.5

Indicate whether the proposed project unnecessarily duplicates experiments conducted by you or other researchers

- ☑ [illegible]
- ☐ [illegible]

5.5.1

(b)(6)

Duplication Search Strategy and Results 5.5.1.1

Address the following two suoparts
Descnbe the strategy you used to determine that the project does not duplicate previous experiments This is usually accomplished by including a descnption and results of a scientific literature review In such a description include the databases searched relevant key search terms and number of hits obtained for each term and combination of terms
In a narrative provide your interpretation/evaluation of the search results
NOTE A table of the search strategy and results may be attached at the end of this document in the ATTACHMENT section Files must be closed before they can be attached

---

5.5.2

Duplication Justification 5.5.2.1

Cite and describe the previous study or studies being duplicated and provide a detailed scientific justification for the duplication

---

Study Design

Study Description 2.1

In the text box below provide a clear descnption of the experimental design and how animals are integrated with expenmental procedures or conditions. Describe or show the assignment of animals to experimental groups or conditions and specify the frequency, duration timing, and/or sequence of procedures
This descnption should allow the IACUC to understand the course of each animal through the study from its arrival/acclimation to euthanasia In this section procedures should be named or described in only general terms. a separate section on Procedures will ask you for procedural details (e g routes of agents anesthesia methods. potential adverse effects etc.).

NOTE Any documents used to illustrate what has been described may be added at the end of the document in the ATTACHMENT section Files must be closed before they can be attached

---

(b)(6)

Animal Numbers, Justification, and Calculations 6.2

In the text box below restate the total number of animals requested (from the section on Authorized Amounts) and show how the total number of animals is calculated, based on group sizes and study design (e.g., 8 animals per group x 5 experimental groups = 40 animals).
Also include a narrative that clearly explains the basis for determining that number using any or all of the following considerations

The numoer of groups needed for a well-controlled expenmental design
The type of biological samples needed from animals relative to the number of animals requested (e g quantity of sample that can be obtained from each animal)
The numoer of animals needed per group based on a statistical requirement, volume/number of biological samples needed or both
NOTE Be as specific as possible For example, if your study design includes multiple doses and time points or multiple control groups, be sure to provide a justification for these additional conditions Any plan to repeat an expenment or include replicates usually is not approved in advance of need If any repetitions or replicates are requested provide the justification below.

Procedures

Use of Standard Operating Procedures (SOPs) 7.1

Will you be using NIOSH-approved SOPs that involve procedures conducted on live animals? Do not include SOPs that do not involve animal procedures

☐ No
☐ Yes

7.1.1

7.1.2

List of SOPs Involving Animal Procedures 7.1.2.1

For each SOP used click the Add Row icon and fill in the requested information Approved SOPs can be cited throughout this protocol form in lieu of describing the procedural details A list of approved SOPs can be found at

(b)(7)(E)

IMPORTANT! SOPs will be reviewed and approved by the IACUC every two (2) years PIs will be notified in the event an SOP is revised. You must always use the most current version of the SOP. If you require any deviation from an approved SOP or latest version of an SOP you must seek IACUC approval before using the procedure. An amendment may be required See the IACUC Administrator for guidance.

SOP Number	SOP Title	SOP Version

Non-Surgical Procedures 7.2

Will non-surgical procedures be performed on live animals? Non-surgical procedures include whole body exposures, nose-only exposure dermal application injections blood sampling identification methods etc performed on live animals NOTE the description of common euthanasia methods are described in a later section

☐ No
☐ Yes

(b)(6)

7.2.1

7.2.2

Description of Non-Surgical Procedures 7.2.2.1

List and describe each non-surgical procedure or set of procedures (if performed at the same time point) that will be performed on live animals. Include the frequency and duration of the procedural treatments. Descriptions must include sufficient detail and a timeline to allow reviewers to understand what the animals will experience and the amount of pain or distress that might be anticipated

---

Adverse Effects of Non-Surgical Procedures 7.2.2.2

Describe any adverse effects or clinical signs expected as a result of the non-surgical procedures. These might include pain, difficulty breathing, tumor development, weight loss, failure to thrive, and seizures, to name a few. In the text box below, organize/group the descriptions and clinical signs by different procedures, experiments, or set of studies, and list the humane endpoints, if applicable, relative to the experimental endpoints

NOTE: Include possible effects or clinical signs that would be seen if inadequate/ineffective doses of anesthesia, analgesia, or sedation are given

---

Plan for Detection and Mitigation of Adverse Effects of Non-Surgical Procedures 7.2.2.3

Provide a plan for how animals will be monitored for pain/distress and the methods used to assess pain/distress. Plans should include frequency and duration of observations during critical periods, interventions (e.g., analgesia, thermal, or nutritional support), and humane endpoints relative to experimental endpoints. Indicate whether any specific metrics/tools will be used (e.g., pain scales, checklists, etc.).

NOTE: All possible adverse effects must be addressed in the plan

---

Non-Survival Surgery 7.3

Will any invasive procedure or surgery be performed in which the animal is euthanized before recovery from anesthesia?

NOTE: Tissue collection or organ perfusion when the animal is unconscious following a fatal dose of euthanasia solution should be described in the separate section on Euthanasia

☐ Yes

☐ No

7.3.1

7.3.2

(b)(6)

Description of Non-Survival Surgery 7.3.2.1

Identify and describe in detail every non-survival surgical procedure. The description should include the surgical approach and the approximate duration of the procedures from anesthesia induction to euthanasia. If helpful, you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section. Be sure to include your anesthetic plan.

Note: The use of aseptic procedures and sterile instruments is recommended. The use of expired materials (e.g., drugs or supplies on live tissues) is prohibited unless specifically described in this section and approved by the IACUC.

Non-Survival Surgery and Responsible Personnel 7.3.2.2

Use the Add Rows icon to list every non-survival surgical procedure and then enter the name all individuals involved in the procedure(s).

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Intraoperative Monitoring during Non-Survival Surgery 7.3.2.3

Describe the steps planned to prevent or minimize pain or distress during the non-survival procedures. Include the plan for monitoring pain and distress, including the frequency and duration. Plans must be developed in consultation with and approved by the Attending Veterinarian.

Survival Surgery 7.4

Will any animal undergo any survival surgery (i.e., be allowed to recover from anesthesia after surgery)?

☐ No

☐ Yes

7.4.1

7.4.2

Description of Survival Surgery 7.4.2.1

Describe every surgery, including preoperative care, aseptic techniques, record keeping, intra-operative monitoring and support, post-operative care, name and qualifications of the surgeon(s). If you purchase surgically manipulated animal models you must have approval from the Attending Veterinarian (and may require an MOU) and must include post-operative care plan in the section below. Include here your anesthetic plan including induction, maintenance and analgesia. Your plan for analgesia should extend through anesthetic recovery and return to housing.
If helpful, you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section. Be sure to include your anesthetic plan.

(b)(6)

Survival Surgery and Responsible Personnel 7.4.2.2

Use the Add Rows icon to list every survival surgical procedure and then enter the name all individuals involved in the procedure(s).

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Post-operative Plan and Support following Survival Surgery 7.4.2.3

Describe the steps planned to prevent or minimize pain or distress during and after survival surgery. Include the plan for monitoring pain and distress, including the frequency and duration. Wound care must be described, and plans should be developed in consultation with and approved by the Attending Veterinarian

Experimental Agents, Drugs, and Substances Used during Procedures 7.6

Use the Add Rows icon to enter every agent, drug, or substance, including euthanasia agents, that will be used during the procedures described above or during any time of study period. For any euthanasia agent, such as pentobarbital or isoflurane, ensure that the dose, route, and schedule, etc., match what is selected or described below in the section on Euthanasia. If necessary, scroll across the page to see all columns.
Note: Use the blue "?" button to the right for appropriate 'Agent Type' categories. Contact IACUC Administrator if additional terms need to be added.
Note: Please also indicate in the previous sections, where appropriate, how and when these agents, drugs, or substances are used in conjunction with the various non-surgical or surgical procedures.

Agent Type (see help)	Name of Agent	Dose or Amount	Route	Frequency or Schedule	Comments

Controlled (CII or CIII) Substances 7.6

Are any of drugs entered above a controlled (CII or CIII) substance? Contact the Attending Veterinarian for guidance.
NOTE: Euthanasia solution containing pentobarbital is a controlled substance and must be listed here.

☐ No

☐ Yes

7.6.1

7.6.2

Controlled Substances 7.6.2.1

For any controlled substance(s) identified above click the Add Rows icon select the substance from the list, and then provide the information requested in the other columns. All persons listed should be named as Protocol Associates above and have training regarding the Controlled Substances Act and local requirements Contact the Attending Veterinarian for assistance.

Name of Controlled Substance(s)	Name of Person(s) with Access	Name of Person(s) that will be dosing	Room Number Where Controlled Drugs will be Stored	Person(s) Responsible for Recordkeeping

Antemortem Sample or Tissue Collection 7.7

Will any biologic or tissue samples be collected from animals before they are euthanized?

- ☐ [illegible]
- ☐ [illegible]

7.7.1

7.7.2

Antemortem Sample or Tissue Collection 7.7.2.1

Use the Add Rows icon to enter the type of tissues or samples to be collected, the amount, volume, or size of sample or tissue collected, the site (body location), and frequency or schedule of sample or tissue collection. If any drugs will be used to alleviate potential pain or distress, be sure to include them in the section on Analgesia Anesthesia and Sedation

Sample or Tissue Type	Amount, Volume, Size	Site	Frequency or Schedule	Other Comments

Genetically Modified (GM) Rodent Use in Experiments 7.8

Check all that apply

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

7.8.1

7.8.2

7.8.3

7.8.4

7.8.5

(b)(6)

Anticipated Adverse Effects in GM Animals 7.8

As a result of the genetic modification, do you anticipate these animals will experience adverse effects above and beyond what is expected in wild type cohorts?
NOTE: Any unanticipated or unexpected proolems or adverse effects that are not explicitly described in the protocol may be suoject to reporting requirements to institutional and regulatory officials and must be reported to the IACUC and AV promptly

☐ Yes
☐ No

7.9.1

7.9.2

Description of Possible or Expected Adverse Effects in GM Animals 7.8.2.1

Describe the possible or expected adverse effects in two parts: (1) Describe all possible or expected adverse effects including any veterinary/housing problems that are possible or expected, and (2) Describe the humane endpoints or criteria for euthanasia if applicable. Lastly, provide any plans for additional supportive care to improve the animal condition that will not interfere with experimental endpoints

Special Concerns for Animal Use

Deviation from Social Housing (ref: IACUC policy) 8.1

Will animals need to be single housed?
NOTE: Single housing may be approved only with a scientific justification or a veterinary-approved behavioral or health reason. The duration of single housing must be minimized, and animals should not be held for extended periods in social isolation awaiting the start of the study. Click the Help icon for more information or contact the IACUC Administrator for guidance

☐ Not Selected
☐ Yes

8.1.1

8.1.2

Justification for Single Housing 8.1.2.1

Consistent with current animal welfare regulations, policies, and AAALAC recommendations, the health and welfare benefits of social housing are enough to consider it the default condition. Therefore, an adequate justification for single housing must include empirical evidence from the scientific literature or experience that supports the conclusion that group housing will negatively affect the study objectives or results. An adequate justification must also weigh the potential adverse effects of single housing. Behavioral and health concerns (e.g., aggression in C57BL/6 male mice) and some restrictive grouping/blocking designs may also justify single housing, but these scenarios also require detailed explanations.

NOTE: For more information, see the IACUC Policy on Social Housing, which can be found here

(b)(6)

Acclimation of Animals after Arrival 8.2

Will animals be acclimated to the facility for at least 5 days after arrival and before procedures are performed?

- ☐ Yes (default)
- ☐ No

8.2.1

8.2.2

Alternative Acclimation Procedure 8.2.2.1

Describe and justify the alternative acclimation procedure

Withholding of Enrichment - Complete this section for all new submissions after September 1, 2017 8.3

See the species-specific enrichment choices below. Conditional items may be introduced with AV and PI approval to address behavioral or health issues.

For Mice:

Default - nesting material (Depending on the strain, crinkle paper may be used alone or in combination with nesting squares.)

Conditional - structures such as shelves to reduce unwanted behaviors such as plugging water bottles, wheels or food treats

For Rats

Default - plastic tubes/tunnels and chew/gnawing substrate (Nylabone or certified wood blocks.)

Conditional - crinkle nesting material with or without nesting squares, kiln dried Aspen shavings instead of wood chip for burrowing or food treats

For Guinea Pigs

Default - shelter

Conditional - food treats

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

8.3.1

8.3.2

8.3.3

8.3.4

Enrichment Restrictions and Plans 8.4

In the text box below: (1) provide scientific justification applicable to your research for providing no enrichment, (2) identify which groups of animals this restriction applies and (3) the plan for how the period of restriction will be minimized.

(b)(6)

Limitations of Enrichment 8.5

Use this section to describe any limitations you have regarding enrichments listed on the species-specific default list above. You must provide a scientific rationale that is applicable to your work.

Regrouping after Acclimation 8.6

Will a regrouping of socially housed animals (change in social group) be required after acclimation or at any time during the study period?

☐ No (default)
☐ Yes

8.6.1

8.6.2

Justification for Regrouping after Acclimation 8.6.2.1

Provide a description and justification for regrouping. Explain why it is necessary and why it will not adversely affect your study objectives.

NOTE: Regrouping animals after acclimation induces significant stress and may lead to aggression/fighting. Methods are available to mitigate this stress. Please contact the Attending Veterinarian for guidance.

Nutritional Restrictions or Additives 8.7

Will the animals receive a non-standard food or water regimen?

☐ No (default)
☐ Yes

8.7.1

8.7.2

Justification for a Non-Standard Food or Water Regimen 8.7.2.1

Address the following three subparts:

1) describe with details the non-standard food or water regimen, including time of day and duration of withdrawal
2) provide a detailed justification
3) describe specific actions to monitor animals for adverse health effects (such as food/water refusal and/or weight loss)

NOTE: Any additives to food or water must be documented in the Study Design and Safety Sections.

(b)(6)

Non-Standard Environment (ref. Satellite Housing policy) 8.8

Will animals be exposed to any environment or condition that is different from the standard home cage environment? Common non-standard environments in our facility include whole body inhalation exposure chambers, metabolic cages plethysmographs environmental chambers, operant chambers, etc

☐ No, [illegible]

☐ Yes

8.8.1

8.8.2

Justification of Non-Standard Environment(s) 8.8.2.1

Address the following subparts

1. describe the non-standard environment
2. provide a detailed justification
3. describe how animals will be acclimated to the non-standard environment
4. describe specific steps taken to monitor animals for adverse health effects
5. for Satellite Facilities provide justification for housing animals outside of the animal facility

NOTE For specialized chambers or animal holding devices, specify details of the environment including its dimensions, air handling and temperature control, duration and frequency of exposures etc If helpful diagrams of the non-standard environment may be attached using the Add Attachments/Links icon

---

Restraint: Prolonged or Non-Routine 8.9

Will animals require prolonged (i.e., greater than a 5 minutes) physical restraint or be confined to a device which prevents normal movement (e.g., nose-only inhalation chambers).
NOTE Momentary manual restraint methods (e.g., handling, Decapicone bags, commercial rodent restrainers) commonly used for drug injections intranasal instillations or similar procedures are not considered prolonged restraint Acclimation to devices and gentle handling prior to the restraint are required to reduce stress in animals Restraint duration should be limited to the minimum that is scientifically necessary. Any observed trauma or distress as a result of the restraint must be promptly reported to the Attending Veterinarian

☐ No

☐ Yes

8.9.1

8.9.2

(b)(6)

Justification for Prolonged or Non-Routine Restraint 8.8.2.1

Address the following four subparts:

1) describe the restraint device or method
2) provide a detailed justification
3) describe how animals will be acclimated to the restraint
4) describe specific steps taken to monitor animals for adverse health effects

NOTE: If helpful, diagrams of the restraint device may be attached in the ATTACHMENT section at the end of this document

---

Multiple Survival Surgeries 8.10

Will multiple survival surgeries be performed on the same animal?

☐ No
☐ Yes

8.10.1

8.10.2

Use of Pharmaceutical Grade Drugs or Agents 8.11

Are all drugs or agents to be used in live animals Pharmaceutical Grade? If there are any exceptions (e.g., if you must use a reagent grade agent or a USP formulation that is not pharmaceutical grade), check No

☐ No
☐ Yes

8.11.1

Description and Justification for Non-Pharmaceutical Grade Drugs or Agents 8.11.1.1

By default, drugs or agent that are used but not included in this table, must be Pharmaceutical Grade. For each non-pharmaceutical grade drug or agent used, click the Add Rows icon and then provide the requested information under each column:

1) name of the drug or agent
2) brief justification for its use (e.g., "pharmaceutical grade is not available")
3) information about the source (e.g., vendor)
4) preparation procedures (e.g., reconstitution, dilution, mixing, final concentrations, aseptic handling, stability, storage)
5) quality control (purity, grade, sterility, pyrogenicity, pH, or other property)

NOTE: Not all USP formulations are pharmaceutical grade; reagents are not pharmaceutical grade compounds. When mixing reagents to be used in live animals, you must describe preparation procedures and, at a minimum, provide information on pH and how you will ensure sterility.

Name of Drug or Agent	Justification	Source	Preparation Procedures	Purity, grade, sterility, pH, pyrogenicity, etc.

(b)(6)

B 11.2

USDA Category E Animals (Unrelieved pain, distress or physiologic impairment) B 12

In the earlier section on Animal Information were any animals assigned to USDA Category E (Category III)?

- ☐ No
- ☐ Yes

B.12.1

B 12.2

USDA Category E (Category III) Scientific Justification and Humane Endpoints B 12.2.1

USDA Category E procedures require additional information. Address each of the following three suoparts:

1. Provide a detailed scientific justification for withholding anesthetics, analgesics, or sedatives
2. Descnoe the steps taken to monrtor the health status of the animals
3. List the humane endpoints (i e , signs or symptoms that will prompt euthanasia or removal from the study)

The Three Rs

Refinement, Replacement, and Reduction B.1

Investigators must show how they have considered alternatives methods and procedures that address animal welfare concerns A consideration of alternatives is usually addressed by responding to the Three Rs (refinement replacement, and reduction)
Refinement - decreasing pain or distress by modifying the husbandry or experimental procedures
Replacement - using methods that avoid or replace the use of animals by substitution with non-animal systems or less sentient animals
Reduction - using strategies that result in fewer animals being used or maximizing the information obtained from the number of animals being used
For help with these concepts and requirements go to http //navc nal usda gov/alternatives or http //vetmed duns duke edu/GLidelinesforAlternativeSearching html or http //altweb jhsph edu

---

Refinement B.2

For each procedure listed in Procedures section that causes pain or distress or has the potential to cause pain or distress descnbe the steps taken to minimize pain or distress or reduce the adverse effects of the procedures.

(b)(6)

Refinement: Supporting Evidence 93

Provide evidence of your consideration of refinements to procedures that may cause pain or distress. Address the following three subparts

:1 List the databases searched (or other sources consulted), the years covered, the key words or search strategy used, and the date of the search. At minimum, the key words should include comprehensive search terms (or MESH terms) for each procedure that has the potential to cause pain or distress and for alternative procedures
:2 Descnoe the search results, including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results.
:3 List and descnbe any additional methods (other than a literature search) that were used in considering refinements (e.g., experts, standards, regulations, conference proceedings, etc.).

NOTE: You may attach a table of the search results in the ATTACHMENT section at the end of this document

---

Replacement 94

Provide the rationale for using animals rather than non-animal alternatives and provide a rationale for the chosen species

---

Replacement: Supporting Evidence 95

Provide evidence of your consideration of replacements for animals. Address the following three subparts

:1 List the databases searched (or other sources consulted), the years covered, the key words or search strategy used, and the date of the search. At minimum, key words should include comprehensive search terms (or MESH terms) for non-animal models and systems (e.g., in vitro, cell culture, computer modeling, simulations, etc.).
:2 Descrioe the search results, including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results.
:3 List and descnbe any additional methods (other than a literature search) that were used in considenng replacements

NOTE: You may attach a table of the search results in the ATTACHMENT section at the end of the document. Files must be closed before they can be attached

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Reduction of Animal Numbers 96

Descnbe the steps taken to reduce, minimize, and/or optimize the number animals being used. Include any statistical plan used to achieve reduction/optimization

NOTE: Reduction refers to efforts that minimize the numbers of animals used. Reduction is addressed by discussing the minimum numbers of animals needed for yielding sufficient samples, the appropriate number animals to achieve statistically significant results, or an experimental design that minimizes the required numoer of animals

---

Euthanasia and Disposition

(b)(6)

Euthanasia Method(s) 10.1

From the list of IACUC-approved methods below, select how the animals will be euthanized as part of the experimental protocol. If multiple methods will be used across different groups of animals or different experiments, describe this in the section on Procedures. Any euthanasia method that deviates from the following specific methods requires the selection of Other and a detailed description.

NOTE: More information about acceptable methods can be found in the NIOSH IACUC policy on Euthanasia and the AVMA Guidelines on Euthanasia

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ Other

10.1.1

10.1.2

10.1.3

10.1.4

Plan Description for Meeting the Euthanasia Method Conditions 10.1.4.1

The AVMA Guidelines for Euthanasia of Animals considers the physical methods descrioed above as "Acceptable with Conditions." As the PI you must provide details on how you will meet those conditions for the method(s) you have chosen. In addition, conscious decapitation requires a scientific justification that explains why this method is required over another accepted method that does not have additional conditions. Please address this in the text box below. See the ACUC Euthanasia policy and SOP for help.

10.1.5

10.1.6

10.1.7

10.1.8

10.1.9

(b)(6)

Other Euthanasia Method 12.1.2.1

Describe the method of euthanasia not listed specifically above. In addition, for any euthanasia method not described as "Acceptable" or "Acceptable with Conditions" in the AVMA Guidelines on Euthanasia, provide a scientific justification. For methods described as "Acceptable with Conditions," describe how the conditions will be met and how training to meet those conditions will be obtained. Please seek a veterinary consult for additional information or guidance.

Disposition of Samples or Tissues Collected Post-Mortem 12.2

Check all that apply. The purpose of this section is to assist with the clearance of publications, posters, and scientific presentations per NIOSH/HELD internal policy, as well as journal requirements for a statement attesting that "animal work was covered by an IACUC approved protocol."
If your intent prior to euthanasia is to share or collect tissue for purposes not within the scope and objectives of this protocol, then you must declare that intent. The IACUC fully supports the concept of tissue sharing and reduction of animal use; however, if there is intent to bank tissues for other uses even though they may be directly or indirectly related to this protocol, then this intent must be documented. If your intent changes during the protocol period, please notify the IACUC to request a change in your answer to this question. (This change can be done administratively.)

Note also:
1. All samples/tissues collected must be identified with a CDC identifier.
2. All samples must be transported consistent with state and federal regulations.
3. All samples that are considered biohazardous may not leave the building without formal approval of the Health and Safety Office.
4. Even with a declaration of intent to share samples or tissue, animals approved under this protocol must not be euthanized for the sole purpose of providing samples or tissues outside of the study objectives without prior IACUC approval.

☐ [illegible]

☐ [illegible]

☐ [illegible]

10.2.1

10.2.2

10.2.3

Safety and Health

Hazardous Agents System Generated 11.1

Using the Add Hazardous Agents icon, select from the list ALL agents (hazardous or not) administered to animals as part of your experiments. The agents listed must match the list of agents provided in the separate hazardous agents attachment described below. If a specific agent does not appear in the list, contact the IACUC Administrator.

Additional Precautions for Animal Care Personnel 11.2

Describe any additional engineering controls, specific instructions for animal husbandry, or specific work practices for animal care personnel.

(b)(6)

Additional Precautions for Laboratory Personnel 11.3

Describe any additional engineering controls specific work practices, or other specific instructions for handling hazardous agents or conducting procedures

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Bedding Disposal Procedures 11.4

Indicate whether routine or standard oedding disposal procedures are acceptable

☐ [illegible]

☐ [illegible]

11.4.1

11.4.2

Other Bedding Disposal Procedures 11.4.2.1

Describe the non-routine or special bedding disposal procedures

---

Special Carcass Removal Procedures 11.5

Indicate whether routine or standard carcass removal procedures are acceptaole

☐ [illegible]

☐ [illegible]

11.5.1

11.5.2

Description of Special Carcass Removal Procedures 11.5.2.1

Describe the non-rouline or special precautions for carcass removal

---

Special Decontamination Procedures 11.6

Indicate whether routine or standard decontamination procedures are acceptable

☐ [illegible]

☐ [illegible]

11.6.1

11.6.2

(b)(6)

Description of Special Decontamination Procedures 11 3 2 1

Describe non-routine or special precautions for decontamination procedures

---

Investigator Assurances & Compliance Agreement

Principal Investigator Assurances and Compliance Agreement 12 1

I will conduct this study in accordance with all applicable rules and regulations as described in the PHS Policy on Humane Care and Use of Laboratory Animals the Federal Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, and CDC/NIOSH Morgantown internal policies (Click the Help icon for links to these resources.)

I will obtain approval from the IACUC before initiating any change in the study objectives, design, or procedures. I understand that work performed without IACUC approval cannot be published with certification of IACUC approval and may result in federally-required reporting of noncompliance

I have determined that the research proposed is not unnecessarily duplicative

I have reviewed the pertinent scientific literature and the sources and/or databases noted in this proposal and found no scientifically acceptable alternatives to any procedures that may cause pain or distress

I confirm that all individuals working on this protocol are enrolled in NIOSH's Occupational Health and Safety Program

I authorize individuals listed on this protocol to conduct procedures involving animals and I accept responsibility for their oversight in the conduct of this proposal. I certify that these same individuals will read, understand and follow the procedures in the final approved version of this protocol and any future amendments.

I confirm that all individuals listed on this protocol have obtained authorization to work with animals in this facility by the Animal Facilities Director or will be required to do so before work with animals begins. Further, I certify that these individuals are properly trained, or will receive such training prior to work with animals in all areas relevant to their assigned work with animals (e.g., biology, handling, and care of the species used, aseptic surgical methods and techniques; the concept of 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; and procedures for reporting animal welfare concerns.)

I certify that all individuals listed on this protocol will complete any additional training, continuing education, or refresher training as assigned by the IACUC

I understand that I am responsible for assuring that my laboratory is in compliance with all federal, state, and local environmental laws and regulations. I accept the responsibility to assure that my laboratory is operated in a safe manner and that all staff and students are informed of potential risks, wear appropriate personal protective equipment, and are adequately trained. I will assure that all personnel working on this protocol receive orientation to laboratory safety instructions, SDS files, and laboratory emergency procedures. I am responsible for adhering to NIOSH safety policies and procedures for handling hazardous materials and for addressing accidental spills and personnel contamination. I will report any significant problems, and research-related accidents and illnesses to the Safety Office, and will complete the required forms in the event of an incident

For animals under this protocol, I accept that in the case of necessary veterinary treatment, the Attending Veterinarian is authorized to provide treatment required to sustain life or, if necessary, provide humane euthanasia to prevent unapproved pain and/or distress. I understand that the animal facility staff will contact me as soon as possible using the emergency contact information that I provide in this protocol, but I understand that such contact may not always be possible prior to treatment or performing euthanasia

I will notify the IACUC of unanticipated adverse outcomes. Unanticipated outcomes are generally defined as negative impacts to animal welfare or well-being that are not explicitly described in the protocol and approved by the IACUC. These include unanticipated outcomes associated with disease, injury, or effects that are under investigation

I accept that veterinary consultation must occur when pain or distress is beyond the level the IACUC approved in the protocol, or when animal facility staff are unable to provide interventional control (i.e., immediate euthanasia.) I will notify the Attending Veterinarian when unanticipated pain or distress, unexpected morbidity, or unanticipated mortality occurs. This applies to animals housed/used at NIOSH and animals funded by NIOSH and housed/used at collaborating institutions.

☐ [illegible]

(b)(6)

12.1.1

ATTACHMENT Section 12.2

Use this section to attach any documents used to illustrate what you have written in the sections above. Keep in mind that attachments are not printed out with the protocol and that the information provided in the protocol must stand on its own.
The IACUC prefers files in pdf images. WORD and Excel files should be saved as pdf before attachment.
Attach the file using the paperclip icon at the right.
For each file attached, complete the description below.

Date Document Added	File Name	File Content/Description	Protocol Sections

For IACUC Administration Use Only

IACUC Admin Section 13.1

This section will be used for the IA to add correspondence or other administrative documents.

☐ [illegible]

☐ N.

13.1.1

13.1.2

Comments 13.2

Appendix 9.3: IACUC Protocol Form

Topaz Annual Renewal Form

Blank Form Report

(b)(6)

Report Comments

FY2019 Annual Protocol Review

Annual Report of Animal Use in FY2019

Protocol Status during the time period of October 1, 2018 - September 30, 2019 1.1

This includes any animal work performed at any facility covered by this protocol (e.g. NIOSH, (b)(4) vendor)
Active means you ordered animals, performed procedures on animals or had animals to carry over from the previous year for ongoing experiments
Inactive means you did not carry animals over from the previous fiscal year, did not order animals during reporting period or did not perform any procedures on live animals.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

1.1.1

1.1.2

1.1.3

Request for Protocol Termination or Closeout 1.2

Complete this section as either N/A or choose termination based on one of the choices provided
Termination/Closeout means you are requesting the protocol be permanently terminated and no further animal work will be permitted. A new protocol will need to be submitted, reviewed and approved to begin additional animal studies

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

1.2.1

1.2.2

1.2.3

1.2.4

(b)(6)

Animal Use during the reporting period 1.3

In each column, report the number of animals which meet the criteria in the column heading. The only animals that will be recorded in more than one column are animals that were housed across fiscal years
Animal Use in this case means animals acquired, dosed, handled, euthanized or died.
Carryover from FY18 means live animals assigned to your protocol as of September 30, 2018 and were still present the following day on October 1, 2018
Vendor source is animals ordered through Topaz from a commercial vendor.
NBP source is animals obtained from the NIOSH Breeding Program
Carryover to FY20 means live animals assigned to your protocol as of September 30, 2019 that were still present the following day on October 1, 2019
Use the green 'plus' sign to add a new line for each pain category and species

Species	Pain Category	Carryoverfrom FY18	Vendor Sourced FY19	NBP Sourced FY19	Carryover toFY20

Animal Disposition during the reporting period 1.4

In each column, report the number of animals for each category. Begin a new row for different species
The Study Endpoint is the time point(s) you described in your study design

Species	Euthanized before Study Endpoint	Died before Study Endpoint	Euthanized at Study Endpoint	Transferred to Another Protocol

Special Activities during reporting period 1.5

Special Activities that occurred during the reporting period (FY19), choose all that apply.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

1.5.1

1.5.2

1.5.3

1.5.4

1.5.5

1.5.6

(b)(6)

1.5.7

1.5.8

1.5.9

1.5.10

NIOSH Breeding Program (NBP) Use 1.6

Summarize use of animals from the NIOSH Breeding Program

☐ N/A - [illegible] from the NBP [illegible]

☐ [illegible] NBP [illegible]

☐ [illegible] NBP [illegible]

1.6.1

1.6.2

1.6.3

NIOSH Breeding Colony Animal Use 1.7

Onsite breeding colonies are both resource rich and inherently wasteful. The PI is required to plan studies to minimize unnecessary production.
In the last column on Proposed Use, provide an estimate of how many animals you plan to use. You will be contacted by the Animal Facility Director for a schedule.

Strain	Sex	Number Requested in the Reporting Year	Number Used in the Reporting Year	Proposed Use in FY20

Unexpected Adverse Events 1.8

An Adverse Event is defined as unexpected death, pain or distress prior to experimental endpoints; equipment failure that impacts animal welfare, pain or distress, or prevents animals from being used to collect experimental data; animal handling accidents such as misinjection, animal escape, animal injury; dosing errors or other similar event that was not documented (described) and approved in this protocol prior to the event occurrence. The IACUC has a policy covering Unexpected Adverse Events. See the policy for more information and guidance.

☐ No adverse events occurred during the reporting period

☐ [illegible] adverse events occurred during the reporting period and are described below

1.8.1

1.8.2

(b)(6)

Describe the unexpected adverse event(s) and the impact on the welfare of your animals. 1.9.2.1

Include date(s), number of animals affected and actions. Note in text box below if you have previously reported this incident to the IACUC during this reporting period.

Corrective and/or Intervention Actions 1.9.2.2

Describe the measures that were taken to minimize or prevent recurrence of those events.

Summary of Anticipated FY20 Animal Use 1.9

Provide a brief summary of the animal use / experiments you plan to conduct in the upcoming fiscal year (FY20).

Continued Animal Use - Future Plans 1.10

Choose the answer that best applies. Look carefully at the choices as the first choice determines if you have completed your annual report or want to continue to modify your protocol in one review step.

- ☐ [illegible]
- ☐ [illegible]

1.10.1

1.10.2

Amendment - Protocol Modifications Section

Protocol Amendment/Modification Summary 2.1

Protocol Amendment / Modification Summary

Each time you create a new amendment, clear all the check marks and click on all that apply for your new request.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

(b)(6)

2.1.1

2.1.2

2.1.3

2.1.4

2.1.5

2.1.6

2.1.7

2.1.8

2.1.9

2.1.10

2.1.11

2.1.12

2.1.13

2.1.14

2.1.15

Overview of Amendment Changes 2.2

Provide an overview or summary of the proposed changes Details must be provided in the pertinent sections below

NOTE Changes should be captured by appending sections below Do not delete previous information that applies to work already performed on animals. Provide a date, experiment number or amendment number as a lead in and identifier for each section Do NOT delete previously approved work as the most recently approved version of the protocol is the official document conveying approval and will be used to establish approval during post approval monitoring

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Administrative Information

Reference Number System Generated 3.1

A unique Reference Number is used only by the TOPAZ database It will not be used to identify the protocol

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(b)(6)

Title — By Item Generated — 3.2

Insert a descriptive protocol title. A good title includes the disease condition, if appropriate, the exposure or agent of interest, and the species. Limit the title to 150 characters.

Protocol Type — By Item Generated — 3.3

Protocol Number — By Item Generated — 3.4

A unique protocol number will be assigned by the IACUC Administrator after approval.

Principal Investigator — By Item Generated — 3.5

Select the Principal Investigator from the list. Do not leave blank; draft versions of the protocol can be saved only if a PI is selected.

Branch or Department — 3.6

Select the Branch or Department of the Principal Investigator

- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]
- ☐ [redacted]

3.6.1

3.6.2

3.6.3

3.6.4

3.6.5

3.6.6

3.6.7

(b)(6)

3.6.6

Other Branch or Department 3.6.1

Enter the Branch or Department of the Principal Investigator

Emergency Contact List 3.7

Use the Add Row icon to enter the name(s) of the Principal Investigator and Protocol Associates authorized to make decisions regarding animal health and welfare. For each individual enter at least one daytime and one after-hours phone number (###-###-####). The first name in the emergency contact list must be the Principal Investigator

Name	Daytime Phone Number	After-Hours Phone Number

Brief Description 3.8

Provide a one(2-3 sentence summary of the study. Indicate the main independent variables, exposure methods, procedures, and general outcomes measures

---

Accounts By Item Generated 3.9

Use the Add Accounts icon to enter the CAN(s) associated with the project. If you do not see the appropriate CAN listed please contact the IACUC Administrator

Funding Source for Purchase of Animals 3.10

Select the source of funding that will be used to purchase animals. (Who will own the animals?)

- [ ] N[illegible]H
- [ ] E[illegible]

3.10.1

3.10.2

Formal Administrative Agreements 3.11

Check YES if any of the animal work described is (or needs to be) covered by a formal agreement such as an Memorandum of Understanding (MOU), Materials Transfer Agreement (MTA), etc
Documentation of these agreement may be attached at the end of this document in the ATTACHMENTS section

- [ ] [illegible]
- [ ] [illegible]

3.11.1

Summary Description of Administrative Agreements 3.11.1.1

(b)(6)

3.11.2

Sensitive or Proprietary Information 3.12

Does this research involve any sensitive or propnetary information that is privileged or confidential?

NOTE: It may not oe necessary to descnbe sensitive or proprietary information in the protocol. Contact the IACUC Administrator for more information and guidance.

☐ No

☐ Yes

3.12.1

3.12.2

Protocol Associates System Generated 3.13

Use the Add Associates icon to select all individuals that will be working on the protocol and their appropnate roles:
Select only individuals who will handle or perform procedures on animals.
Animal & Inhalation Facility Contractors and Some Histopathology Personnel should be added as a group. Scroll down to 'Staff' in the personnel list to identify these groups as needed. The IACUC will monitor the training for these individuals.
Contact the IACUC Administrator for a list of pre-qualified individuals if you have questions.

In the "Responsibilities" text field below each added Protocol Associate, list all specific procedures (e.g., specific non-surgical procedures (e.g., oropharyngeal aspiration, intraperitoneal injection, anesthesia by open-drop isoflurane anesthesia with ketamine cocktail); survival and non-survival surgeries (e.g., surgical placement of intra-abdominal transmitter, sub-cutaneous implant of osmotic pump, invasive pleythsmography through tracheotomy, jugular/carotid cut down), euthanasia (e.g., barbiturate overdose and thoracotomy), etc.) that the individual will perform on animals. You must include sufficient detail that training and expenence to perform the procedure can be assessed oy the IACUC.

Co-Investigator: Co-Investigators have the same TOPAZ Elements access and editing privileges as the Pnmary Investigator.
Key Associate: Allows the individual to receive protocol related email messages but can only view the protocol in TOPAZ Elements.
Authorized to Order Animals: Allows the individual to place animal orders for the protocol in TOPAZ Elements.
Role Not Checked: Individual can only view the protocol in TOPAZ Elements.
NOTE: If an individual's name does not appear in the database, the individual may require training and authonzation to work with animals. Contact the IACUC Administrator for more information and guidance on adding individuals to the dataoase.

Personnel Training and Experience 3.14

Check whether the Training and Experience Forms for the PI and every Protocol Associate are current and up-to-date.
Please consult with the IACUC Administrator for guidance on training and experience requirements. Any individual, including the PI, who has not met or completed all IACUC training and experience requirements is not authonzed to perform animal work under this protocol.
Note: Blank training and experience forms and all existing experience forms can be found at
(b)(7)(E) It is the PI's responsibility to ensure that training forms for all protocol associates are kept up-to-date and saved in the shared folder.

☐ [illegible]

3.14.1

Animal Information

(b)(6)

Species 4.1

Use the "Add Species" icon to select the species of animal. Only one species per protocol is allowed.
Note: Some fields in this section may be hidden. Be sure to select the Expand All Items icon in the outline to see all relevant fields.

4.1.1

Mouse

Vendor and Strain/Stock/Breed System Generated 4.1.1.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor | Strain/Stock/Breed | Vendor Strain/Stock/Breed Link

Authorized Amounts System Generated 4.1.1.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

Additional Animal Information 4.1.1.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon.

Sex	Age	Weight	Additional Features, Instructions, or Comments

---

4.1.2

Guinea Pig

Vendor and Strain/Stock/Breed System Generated 4.1.2.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain, stock, or breed is not listed, please contact the IACUC Administrator.

Vendor | Strain/Stock/Breed | Vendor Strain/Stock/Breed Link

Authorized Amounts System Generated 4.1.2.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section.

(b)(6)

Additional Animal Information 4.1.2.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon

Sex	Age	Weight	Additional Features, Instructions, or Comments

---

4.1.3

Mouse

Vendor and Strain/Stock/Breed System Generated 4.1.3.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain stock, or breed is not listed, please contact the IACUC Administrator

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts System Generated 4.1.3.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section

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Additional Animal Information 4.1.3.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rows icon

Sex	Age	Weight	Additional Features, Instructions, or Comments

---

4.1.4

Rat

Vendor and Strain/Stock/Breed System Generated 4.1.4.1

Use the Add Vendor and Strain/Stock/Breed icon to add each type of animal requested. If your preferred vendor, strain stock, or breed is not listed, please contact the IACUC Administrator

Vendor	Strain/Stock/Breed	Vendor Strain/Stock/Breed Link

Authorized Amounts System Generated 4.1.4.2

Use the Add Pain Categories icon to enter the total number of animals needed under each appropriate USDA pain category B, C, D or E. Additional questions about how you determined the animal numbers will be asked in another section

---

(b)(6)

Additional Animal Information 4.1.4.3

Enter additional animal information that will be used for ordering/breeding/handling. Additional rows can be added by clicking the Add Rowsicon

Sex	Age	Weight	Additional Features, Instructions, or Comments

Breeding Colony 4.2

Will you need to maintain a breeding colony? NOTE: IACUC protocol approval does not imply availability of resources (space and costs) for maintaining on site breeding colonies

- [ ] No
- [ ] Yes

4.2.1

4.2.2

Justification for a Breeding Colony 4.2.2.1

Justify the need to maintain a breeding colony in the text box below. See the IACUC Breeding Colony policy and complete a breeding roadmap and updates as requested by the holder of the approved IACUC breeding protocol

Animal Identification 4.3

Select the primary method that you will use for identifying animals. Cage cards will be placed by the Animal Facility

- [ ] [illegible]
- [ ] [illegible]
- [ ] Tattoo
- [ ] [illegible]
- [ ] [illegible]

4.3.1

4.3.2

4.3.3

4.3.4

4.3.5

(b)(6)

Other Method of Animal Identification 4.3.6.1

List or briefly describe the other method for identifying animals. If the method involves an invasive procedure or has the potential to cause pain or distress, also describe this method in detail in the Procedures section.

Animal Housing and Use Areas

Animal Housing Location(s) 5.1

Check the location(s) where animals will be housed. "Housing" refers to locations in which animals are kept for 12 hours or more. A separate question will ask about the location(s) where procedures will be performed on animals.

- ☐ NIOSH Facility
- ☐ Off-Site Location(s)

5.1.1

Preferred NIOSH Housing Location(s) 5.1.1.1

Click the Add Rows icon to list the specific room locations where you wish to house animals. Also enter any additional features, equipment, or environmental requirements and the duration of housing.

Building/Area/Room Number	Other requirements (e.g., fume hood, biosafety cab	Housing Duration

---

5.1.2

Off-Site Housing Location(s) 5.1.2.1

Use the Add Row icon to list every off-site animal housing location in the table below. A brief justification and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information.

Institution/Department	Building/Room Number	Housing Durations	Brief Justification	AAALAC Accredited?	PHS Assurance Number

---

Individual(s) Responsible for Off-Site Housing 5.1.2.2

Enter the name of the individual responsible for the animals while they are housed off-site. Include emergency contact information (e.g., department, office, email, and day/evening phone numbers).

Animal Procedures Location(s) 5.2

Check the location(s) where procedures will be performed on live animals.

- ☐ NIOSH Animal Facility or Laboratory Area
- ☐ Off-Site Location(s)

5.2.1

(b)(6)

Location(s) of Animal Procedures and/or Drugs Intended for Animals 5.2.1.1

Click the Add Rows icon to list the specific NIOSH room locations where you wish to perform procedures on animals and where drugs intended for use in animals are kept. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) performed at each location.

NOTE: Include room locations where pharmaceutical drugs or other agents intended for use in animals will be stored, even if no other procedures are performed there.

Room/Lab Number	List of Procedures	Are drugs or agents stored here?

5.2.2

Off-Site Procedures Location(s) 5.2.2.1

Use the Add Row icon to list every off-site location where procedures will be conducted. List all procedures (e.g., euthanasia, intratracheal instillations, tail blood collection, restraint, etc.) that will be performed on live animals at this off-site location. A brief justification for requiring off-site procedures and information about AAALAC Accreditation and PHS Assurance are required. Click the Help icon for more information.

Institution/Department	Building/Room Number	List of	Brief Justification	AAALAC Accredited?	PHS Assurance Number

Individual(s) Responsible for Off-Site Procedures 5.2.2.2

Enter the name of the individual(s) responsible for conducting procedures on animals while they are used off-site. Include emergency contact information (e.g., department, office, email, and day and evening phone numbers).

Transport of Animals 5.3

Check how animals will be transported within the NIOSH facility or to and from other off-site locations. Check all that apply.

- ☐ [illegible]
- ☐ [illegible] (b)(4)
- ☐ [illegible]
- ☐ [illegible]

5.3.1

5.3.2

5.3.3

(b)(6)

Animal Transport by the PI, Laboratory Staff, or Other Personnel 5.3.3.1

Describe how animals will be transported within the NIOSH facility, and name who will be responsible for transporting the animals. If available, attach copies of any SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

5.3.4

Animal Transport to or from an Off-Site Location 5.3.4.1

Describe how animals will be transported to or from an off-site location, and name who will be responsible for transporting the animals. If available, attach copies of any SOP or other formal agreement pertaining to the transport procedures to the Administrative Information section under Administrative Attachments.

Study Objectives and Scientific Merit

Study Aims, Objectives, and Scientific Rationale 6.1

In the text field below, explain the specific aims, objectives, and scientific rationale of the study. Do not simply cut and paste from a scientific research proposal. The narrative should be written in language understandable to an educated layperson.

Role of Animals 6.2

In the text field below, briefly describe the role of animals in meeting the study objectives.
NOTE: You will also be asked to address alternatives to the use of animals in a separate section on the 3R's (refinement, reduction, replacement).

Potential Impact 6.3

In the text box below, explain the impact or benefit (e.g., health, safety, or the good of society) to be derived from the study.

Scientific Merit 6.4

How has the scientific merit of this study been evaluated? Check all that apply.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

6.4.1

(b)(6)

6.4.2

6.4.3

6.4.4

Other Method for Evaluating Scientific Merit or Additional Information 6.4.4.1

Briefly describe the other method(s) used to evaluate the scientific merit of the project

---

Duplication Assurance 6.5

Indicate whether the proposed project unnecessarily duplicates experiments conducted by you or other researchers

☑ [illegible]

☐ [illegible]

6.5.1

Duplication Search Strategy and Results 6.5.1.1

Address the following two subparts
Describe the strategy you used to determine that the project does not duplicate previous experiments This is usually accomplished by including a description and results of a scientific literature review In such a description include the databases searched relevant key search terms and number of hits obtained for each term and combination of terms In a narrative provide your interpretation/evaluation of the search results
NOTE A table of the search strategy and results may be attached at the end of this document in the ATTACHMENT section Files must be closed before they can be attached

---

6.5.2

Duplication Justification 6.5.2.1

Cite and describe the previous study or studies being duplicated and provide a detailed scientific justification for the duplication

---

Study Design

(b)(6)

Study Description 7.1

In the text box below, provide a clear description of the experimental design and how animals are integrated with experimental procedures or conditions. Describe or show the assignment of animals to experimental groups or conditions, and specify the frequency, duration, timing, and/or sequence of procedures.
This description should allow the IACUC to understand the course of each animal through the study, from its arrival/acclimation to euthanasia. In this section, procedures should be named or described in only general terms, a separate section on Procedures will ask you for procedural details (e.g., routes of agents, anesthesia methods, potential adverse effects, etc.).

NOTE: Any documents used to illustrate what has been described may be added at the end of the document in the ATTACHMENT section. Files must be closed before they can be attached.

Animal Numbers, Justification, and Calculations 7.2

In the text box below, restate the total number of animals requested (from the section on Authorized Amounts), and show how the total number of animals is calculated, based on group sizes and study design (e.g., 8 animals per group x 5 experimental groups = 40 animals).
Also include a narrative that clearly explains the basis for determining that number using any or all of the following considerations:

The number of groups needed for a well-controlled experimental design.
The type of biological samples needed from animals, relative to the number of animals requested (e.g., quantity of sample that can be obtained from each animal).
The number of animals needed per group based on a statistical requirement, volume/number of biological samples needed, or both.
NOTE: Be as specific as possible. For example, if your study design includes multiple doses and time points or multiple control groups, be sure to provide a justification for these additional conditions. Any plan to repeat an experiment or include replicates usually is not approved in advance of need. If any repetitions or replicates are requested, provide the justification below:

Procedures

Use of Standard Operating Procedures (SOPs) 8.1

Will you be using NIOSH-approved SOPs that involve procedures conducted on live animals? Do not include SOPs that do not involve animal procedures.

☐ No

☐ Yes

8.1.1

8.1.2

(b)(6)

List of SOPs Involving Animal Procedures 3.1.3.1

For each SOP used, click the Add Row icon and fill in the requested information. Approved SOPs can be cited throughout this protocol form in lieu of describing the procedural details. A list of approved SOPs can be found at

(b)(7)(E)

IMPORTANT! SOPs will be reviewed and approved by the IACUC every two (2) years. PIs will be notified in the event an SOP is revised. You must always use the most current version of the SOP. If you require any deviation from an approved SOP or latest version of an SOP, you must seek IACUC approval before using the procedure. An amendment may be required. See the IACUC Administrator for guidance.

SOP Number	SOP Title	SOP Version

Non-Surgical Procedures 3.2

Will non-surgical procedures be performed on live animals? Non-surgical procedures include whole body exposures, nose-only exposure, dermal application, injections, blood sampling, identification methods, etc., performed on live animals. NOTE: the description of common euthanasia methods are described in a later section.

- ☐ No
- ☐ Yes

3.2.1

3.2.2

Description of Non-Surgical Procedures 3.2.2.1

List and describe each non-surgical procedure or set of procedures (if performed at the same time point) that will be performed on live animals. Include the frequency and duration of the procedural treatments. Descriptions must include sufficient detail and a timeline to allow reviewers to understand what the animals will experience and the amount of pain or distress that might be anticipated.

Adverse Effects of Non-Surgical Procedures 3.2.2.2

Describe any adverse effects or clinical signs expected as a result of the non-surgical procedures. These might include pain, difficulty breathing, tumor development, weight loss, failure to thrive, and seizures, to name a few. In the text box below, organize/group the descriptions and clinical signs by different procedures, experiments, or set of studies, and list the humane endpoints, if applicable, relative to the experimental endpoints.

NOTE: Include possible effects or clinical signs that would be seen if inadequate/ineffective doses of anesthesia, analgesia, or sedation are given.

(b)(6)

Plan for Detection and Mitigation of Adverse Effects of Non-Surgical Procedures 8.2.2.3

Provide a plan for how animals will be monitored for pain/distress and the methods used to assess pain/distress Plans should include frequency and duration of observations during critical periods, interventions (e g analgesia thermal, or nutritional support) and humane endpoints relative to experimental endpoints Indicate whether any specific metrics/tools will be used (e g pain scales, checklists etc).

NOTE All possible adverse effects must be addressed in the plan

Non-Survival Surgery 8.3

Will any invasive procedure or surgery be performed in which the animal is euthanized before recovery from anesthesia?

NOTE Tissue collection or organ perfusion when the animal is unconscious following a fatal dose of euthanasia solution should be described in the separate section on Euthanasia

☐ No

☐ Yes

8.3.1

8.3.2

Description of Non-Survival Surgery 8.3.2.1

Identify and describe in detail every non-survival surgical procedure The description should include the surgical approach and the approximate duration of the procedures from anesthesia induction to euthanasia If helpful you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section. Be sure to include your anesthetic plan

Note The use of aseptic procedures and sterile instruments is recommended The use of expired materials (e g drugs or supplies on live tissues) is prohibited unless specifically described in this section and approved by the IACUC.

Non-Survival Surgery and Responsible Personnel 8.3.2.2

Use the Add Rows icon to list every non-survival surgical procedure and then enter the name all individuals involved in the procedure(s).

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Intraoperative Monitoring during Non-Survival Surgery 8.3.2.3

Describe the steps planned to prevent or minimize pain or distress during the non-survival procedures Include the plan for monitoring pain and distress including the frequency and duration Plans must be developed in consultation with and approved by the Attending Veterinarian

(b)(6)

Survival Surgery 8.4

Will any animal undergo any survival surgery (i.e., be allowed to recover from anesthesia after surgery)?

☐ No

☐ Yes

8.4.1

8.4.2

Description of Survival Surgery 8.4.2.1

Describe every surgery, including preoperative care, aseptic techniques, record keeping, intra-operative monitoring and support, post-operative care, name and qualifications of the surgeon(s). If you purchase surgically manipulated animal models you must have approval from the Attending Veterinarian (and may require an MOU), and must include post-operative care plan in the section below. Include here your anesthetic plan including induction, maintenance and analgesia. Your plan for analgesia should extend through anesthetic recovery and return to housing.
If helpful, you may add pictures or diagrams to illustrate the procedures at the end of this document in the ATTACHMENT section. Be sure to include your anesthetic plan.

Survival Surgery and Responsible Personnel 8.4.2.2

Use the Add Rows icon to list every survival surgical procedure and then enter the name all individuals involved in the procedure(s).

Name of Procedure(s)	Name of Surgeon(s)	Name of Anesthetist(s)

Post-operative Plan and Support Following Survival Surgery 8.4.2.3

Describe the steps planned to prevent or minimize pain or distress during and after survival surgery. Include the plan for monitoring pain and distress, including the frequency and duration. Wound care must be described, and plans should be developed in consultation with and approved by the Attending Veterinarian.

Experimental Agents, Drugs, and Substances Used during Procedures 8.5

Use the Add Rows icon to enter every agent, drug, or substance, including euthanasia agents, that will be used during the procedures described above or during any time of study period. For any euthanasia agent, such as pentobarbital or isoflurane, ensure that the dose, route, and schedule, etc., match what is selected or described below in the section on Euthanasia. If necessary, scroll across the page to see all columns.
Note: Use the blue "?" button to the right for appropriate 'Agent Type' categories. Contact IACUC Administrator if additional terms need to be added.
Note: Please also indicate in the previous sections, where appropriate, how and when these agents, drugs, or substances are used in conjunction with the various non-surgical or surgical procedures.

Agent Type (see help)	Name of Agent	Dose or Amount	Route	Frequency or Schedule	Comments

---

(b)(6)

Controlled (CII or CIII) Substances 8.6

Are any of drugs entered above a controlled (CII or CIII) substance? Contact the Attending Vetennanan for guidance
NOTE Euthanasia solution containing pentobarbital is a controlled suostance and must be listed here

- ☐ [illegible]
- ☐ [illegible]

8.6.1

8.6.2

Controlled Substances 8.6.2.1

For any controlled substance(s) identified above click the Add Rows icon select the substance from the list, and then provide the information requested in the other columns All persons listed should be named as Protocol Associates above and have training regarding the Controlled Suostances Act and local requirements Contact the Attending Vetennanan for assistance

Name of Controlled Substance(s)	Name of Person(s) with Access	Name of Person(s) that will be dosing	Room Number Where Controlled Drugs will be Stored	Person(s) Responsible for Recordkeeping

---

Antemortem Sample or Tissue Collection 8.7

Will any biologic or tissue samples be collected from animals before they are euthanized?

- ☐ [illegible]
- ☐ [illegible]

8.7.1

8.7.2

Antemortem Sample or Tissue Collection 8.7.2.1

Use the Add Rows icon to enter the type of tissues or samples to be collected, the amount, volume, or size of sample or tissue collected, the site (body location), and frequency or schedule of sample or tissue collection. If any drugs will be used to alleviate potential pain or distress, be sure to include them in the section on Analgesia Anesthesia and Sedation

Sample or Tissue Type	Amount, Volume, Size	Site	Frequency or Schedule	Other Comments

---

Genetically Modified (GM) Rodent Use in Experiments 8.8

Check all that apply

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

8.8.1

(b)(6)

8.8.2

8.8.3

8.8.4

8.8.5

Anticipated Adverse Effects in GM Animals 8.9

As a result of the genetic modification, do you anticipate these animals will experience adverse effects above and beyond what is expected in wild type cohorts?
NOTE: Any unanticipated or unexpected problems or adverse effects that are not explicitly described in the protocol may be subject to reporting requirements to institutional and regulatory officials and must be reported to the ACUC and AV promptly.

☐ No

☐ Yes

8.9.1

8.9.2

Description of Possible or Expected Adverse Effects in GM Animals 8.9.2.1

Describe the possible or expected adverse effects in two parts: (1) Describe all possible or expected adverse effects including any veterinary/housing problems that are possible or expected, and (2) Describe the humane endpoints or criteria for euthanasia if applicable. Lastly, provide any plans for additional supportive care to improve the animal condition that will not interfere with experimental endpoints.

Special Concerns for Animal Use

Deviation from Social Housing (ref. IACUC policy) 9.1

Will animals need to be single housed?
NOTE: Single housing may be approved only with a scientific justification or a veterinary-approved behavioral or health reason. The duration of single housing must be minimized, and animals should not be held for extended periods in social isolation awaiting the start of the study. Click the Help icon for more information or contact the IACUC Administrator for guidance.

☐ No Selection

☐ Yes

9.1.1

9.1.2

(b)(6)

Justification for Single Housing 9.1.2.1

Consistent with current animal welfare regulations, policies, and AAALAC recommendations, the health and welfare benefits of social housing are enough to consider it the default condition. Therefore, an adequate justification for single housing must include empirical evidence from the scientific literature or experience that supports the conclusion that group housing will negatively affect the study objectives or results. An adequate justification must also weigh the potential adverse effects of single housing. Behavioral and health concerns (e.g., aggression in C57BL/6 male mice) and some restrictive grouping/blocking designs may also justify single housing, but these scenarios also require detailed explanations.

NOTE: For more information, see the IACUC Policy on Social Housing, which can be found here.

Acclimation of Animals after Arrival 9.2

Will animals be acclimated to the facility for at least 5 days after arrival and before procedures are performed?

- ☐ Yes (default)
- ☐ No

9.2.1

9.2.2

Alternative Acclimation Procedure 9.2.2.1

Describe and justify the alternative acclimation procedure.

Withholding of Enrichment - Complete this section for all new submissions after September 1, 2017 9.3

See the species-specific enrichment choices below. Conditional items may be introduced with AV and PI approval to address behavioral or health issues.

For Mice:

Default - nesting material (Depending on the strain, crinkle paper may be used alone or in combination with nesting squares.)

Conditional - structures such as shelves to reduce unwanted behaviors such as plugging water bottles; wheels or food treats

For Rats

Default - plastic tubes/tunnels and chew/gnawing substrate (Nylabone or certified wood blocks).

Conditional - crinkle nesting material with or without nesting squares. Kiln dried Aspen shavings instead of wood chip for burrowing or food treats

For Guinea Pigs:

Default - shelter

Conditional - food treats

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]

9.3.1

(b)(6)

9.3.2

9.3.3

9.3.4

Enrichment Restrictions and Plans 9.4

In the text box below: (1) provide scientific justification applicable to your research for providing no enrichment (2) identify which groups of animals this restriction applies and (3) the plan for how the period of restriction will be minimized

---

Limitations of Enrichment 9.6

Use this section to describe any limitations you have regarding enrichments listed on the species-specific default list above. You must provide a scientific rationale that is applicable to your work

---

Regrouping after Acclimation 9.8

Will a regrouping of socially housed animals (change in social group) be required after acclimation or at any time during the study period?

- ☐ No Default
- ☐ Yes

9.8.1

9.8.2

Justification for Regrouping after Acclimation 9.8.2.1

Provide a description and justification for regrouping. Explain why it is necessary and why it will not adversely affect your study objectives

NOTE: Regrouping animals after acclimation induces significant stress and may lead to aggression/fighting. Methods are available to mitigate this stress. Please contact the Attending Veterinarian for guidance

---

Nutritional Restrictions or Additives 9.7

Will the animals receive a non-standard food or water regimen?

- ☐ No Default
- ☐ Yes

9.7.1

9.7.2

(b)(6)

Justification for a Non-Standard Food or Water Regimen 8.7.2.1

Address the following three subparts:

(1) describe with details the non-standard food or water regimen including time of day and duration of withdrawal
(2) provide a detailed justification
(3) describe specific actions to monitor animals for adverse health effects (such as food/water refusal and/or weight loss)

NOTE: Any additives to food or water must be documented in the Study Design and Safety Sections

---

Non-Standard Environment (ref. Satellite Housing policy) 8.8

Will animals be exposed to any environment or condition that is different from the standard home cage environment? Common non-standard environments in our facility include whole body inhalation exposure chambers, metabolic cages, plethysmographs, environmental chambers, operant chambers, etc.

☐ No (skip)

☐ Yes

9.8.1

9.8.2

Justification of Non-Standard Environment(s) 8.8.2.1

Address the following subparts:

(1) describe the non-standard environment
(2) provide a detailed justification
(3) describe how animals will be acclimated to the non-standard environment
(4) describe specific steps taken to monitor animals for adverse health effects
(5) for Satellite Facilities, provide justification for housing animals outside of the animal facility

NOTE: For specialized chambers or animal holding devices, specify details of the environment including its dimensions, air handling and temperature control, duration and frequency of exposures, etc. If helpful, diagrams of the non-standard environment may be attached using the Add Attachments/Links icon.

Restraint: Prolonged or Non-Routine 9.9

Will animals require prolonged (i.e., greater than a 5 minutes) physical restraint or be confined to a device which prevents normal movement (e.g., nose-only inhalation chambers)?
NOTE: Momentary manual restraint methods (e.g., handling, Decapicone bags, commercial rodent restrainers) commonly used for drug injections, intranasal instillations, or similar procedures are not considered prolonged restraint. Acclimation to devices and gentle handling prior to the restraint are required to reduce stress in animals. Restraint duration should be limited to the minimum that is scientifically necessary. Any observed trauma or distress as a result of the restraint must be promptly reported to the Attending Veterinarian.

☐ No

☐ Yes

(b)(6)

9.9.1

9.9.2

Justification for Prolonged or Non-Routine Restraint 9.9.2.1

Address the following four subparts:

1. describe the restraint device or method
2. provide a detailed justification
3. describe how animals will be acclimated to the restraint
4. describe specific steps taken to monitor animals for adverse health effects

NOTE: If helpful, diagrams of the restraint device may be attached in the ATTACHMENT section at the end of this document

---

Multiple Survival Surgeries 9.10

Will multiple survival surgeries be performed on the same animal?

☐ No

☐ Yes

9.10.1

9.10.2

Use of Pharmaceutical Grade Drugs or Agents 9.11

Are all drugs or agents to be used in live animals Pharmaceutical Grade? If there are any exceptions (e.g., if you must use a reagent grade agent or a USP formulation that is not pharmaceutical grade), check No

☐ No

☐ Yes

9.11.1

(b)(6)

Description and Justification for Non-Pharmaceutical Grade Drugs or Agents 9.11.1

By default, drugs or agent that are used, but not included in this table, must be Pharmaceutical Grade. For each non-pharmaceutical grade drug or agent used, click the Add Rows icon and then provide the requested information under each column:

1. name of the drug or agent
2. brief justification for its use (e.g., "pharmaceutical grade is not available");
3. information about the source (e.g., vendor);
4. preparation procedures (e.g., reconstitution, dilution, mixing, final concentrations, aseptic handling, stability, storage);
5. quality control (purity, grade, sterility, pyrogenicity, pH, or other property).

NOTE: Not all USP formulations are pharmaceutical grade; reagents are not pharmaceutical grade compounds. When mixing reagents to be used in live animals, you must describe preparation procedures and, at a minimum, provide information on pH and how you will ensure sterility.

Name of Drug or Agent	Justification	Source	Preparation Procedures	Purity, grade, sterility, pH, pyrogenicity, etc.

9.11.2

USDA Category E Animals (Unrelieved pain, distress or physiologic impairment) 9.12

In the earlier section on Animal Information, were any animals assigned to USDA Category E (Category III)?

- ☐ No
- ☐ Yes

9.12.1

9.12.2

USDA Category E (Category III) Scientific Justification and Humane Endpoints 9.12.3.1

USDA Category E procedures require additional information. Address each of the following three subparts:

1. Provide a detailed scientific justification for withholding anesthetics, analgesics, or sedatives
2. Describe the steps taken to monitor the health status of the animals
3. List the humane endpoints (i.e., signs or symptoms that will prompt euthanasia or removal from the study).

The Three Rs

(b)(6)

Refinement, Replacement, and Reduction 10.1

Investigators must show how they have considered alternatives methods and procedures that address animal welfare concerns. A consideration of alternatives is usually addressed by responding to 'the Three Rs' (refinement, replacement, and reduction)
Refinement - decreasing pain or distress by modifying the husbandry or experimental procedures
Replacement - using methods that avoid or replace the use of animals by substitution with non-animal systems or less sentient animals
Reduction - using strategies that result in fewer animals being used or maximizing the information obtained from the number of animals being used
For help with these concepts and requirements, go to http://awic.nal.usda.gov/alternatives or http://vetmed.duhs.duke.edu/GuidelinesforAlternativeSearching.html or http://altweb.jhsph.edu

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Refinement 10.2

For each procedure listed in Procedures section that causes pain or distress or has the potential to cause pain or distress, describe the steps taken to minimize pain or distress or reduce the adverse effects of the procedures

---

Refinement, Supporting Evidence 10.3

Provide evidence of your consideration of refinements to procedures that may cause pain or distress. Address the following three subparts

(1) List the databases searched (or other sources consulted), the years covered, the key words or search strategy used and the date of the search. At minimum, the key words should include comprehensive search terms (or MESH terms) for each procedure that has the potential to cause pain or distress and for alternative procedures
(2) Describe the search results, including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results
(3) List and describe any additional methods (other than a literature search) that were used in considering refinements (e.g., experts, standards, regulations, conference proceedings, etc.).

NOTE: You may attach a table of the search results in the ATTACHMENT section at the end of this document

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Replacement 10.4

Provide the rationale for using animals rather than non-animal alternatives and provide a rationale for the chosen species.

(b)(6)

Replacement: Supporting Evidence 12.5

Provide evidence of your consideration of replacements for animals. Address the following three subparts:

1. List the databases searched (or other sources consulted), the years covered, the key words or search strategy used and the date of the search. At minimum, key words should include comprehensive search terms (or MESH terms) for non-animal models and systems (e.g., in vitro, cell culture, computer modeling, simulations, etc.).
2. Describe the search results, including the number of hits for relevant search terms and a narrative summarizing your interpretation/evaluation of the results.
3. List and describe any additional methods (other than a literature search) that were used in considering replacements.

NOTE: You may attach a table of the search results in the ATTACHMENT section at the end of the document. Files must be closed before they can be attached.

Reduction of Animal Numbers 10.8

Describe the steps taken to reduce, minimize, and/or optimize the number animals being used. Include any statistical plan used to achieve reduction/optimization.

NOTE: Reduction refers to efforts that minimize the numbers of animals used. Reduction is addressed by discussing the minimum numbers of animals needed for yielding sufficient samples, the appropriate number animals to achieve statistically significant results, or an experimental design that minimizes the required number of animals.

Euthanasia and Disposition

Euthanasia Method(s) 11.1

From the list of IACUC-approved methods below, select how the animals will be euthanized as part of the experimental protocol. If multiple methods will be used across different groups of animals or different experiments, describe this in the section on Procedures. Any euthanasia method that deviates from the following specific methods requires the selection of Other and a detailed description.
NOTE: More information about acceptable methods can be found in the NIOSH IACUC policy on Euthanasia and the AVMA Guidelines on Euthanasia.

- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ [illegible]
- ☐ Other

11.1.1

11.1.2

(b)(6)

11 1.3

11.1.4

Plan Description for Meeting the Euthanasia Method Conditions 111.4.1

The AVMA Guidelines for Euthanasia of Animals considers the physical methods descrioed above as "Acceptable with Conditions." As the PI you must provide details on how you will meet those conditions for the methods you have chosen In addition, conscious decapitation requires a scientific justification that explains why this method is required over another accepted method that does not have additional conditions Please address this in the text box oelow See the ACUC Euthanasia policy and SOP for help

---

11.1.5

11.1.6

11.1.7

11.1.8

11.1.9

Other Euthanasia Method 11 1 5 1

Descnbe the method of euthanasia not listed specifically aoove In addition for any euthanasia method not descnoed as 'Acceptaole' or "Acceptable with Conditions' in the AVMA Guidelines on Euthanasia, provide a scientific justification For methods described as "Acceptable with Condilions " describe how the conditions will be met and how training to meet those conditions will be obtained Please seek a vetennary consult for addrtional information or guidance

(b)(6)

Disposition of Samples or Tissues Collected Post-Mortem 11.3

Check all that apply. The purpose of this section is to assist with the clearance of publications, posters, and scientific presentations per NIOSH/HELD internal policy, as well as journal requirements for a statement attesting that "animal work was covered by an IACUC approved protocol."
If your intent prior to euthanasia is to share or collect tissue for purposes not within the scope and objectives of this protocol, then you must declare that intent. The IACUC fully supports the concept of tissue sharing and reduction of animal use. However, if there is intent to bank tissues for other uses even though they may be directly or indirectly related to this protocol, then this intent must be documented. If your intent changes during the protocol period, please notify the IACUC to request a change in your answer to this question. (This change can be done administratively.)

Note also
1. All samples/tissues collected must be identified with a CDC identifier
2. All samples must be transported consistent with state and federal regulations
3. All samples that are considered biohazardous may not leave the building without formal approval of the Health and Safety Office
4. Even with a declaration of intent to share samples or tissue, animals approved under this protocol must not be euthanized for the sole purpose of providing samples or tissues outside of the study objectives without prior IACUC approval

☐ [illegible] 11.2.1

☐ [illegible] 11.2.2

☐ [illegible] 11.2.3

Safety and Health

Hazardous Agents System Generated 12.1

Using the Add Hazardous Agents icon, select from the list ALL agents (hazardous or not) administered to animals as part of your experiments. The agents listed must match the list of agents provided in the separate hazardous agents attachment described below. If a specific agent does not appear in the list, contact the IACUC Administrator.

Additional Precautions for Animal Care Personnel 12.2

Describe any additional engineering controls, specific instructions for animal husbandry, or specific work practices for animal care personnel

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Additional Precautions for Laboratory Personnel 12.3

Describe any additional engineering controls, specific work practices, or other specific instructions for handling hazardous agents or conducting procedures

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(b)(6)

Bedding Disposal Procedures 12.4

Indicate whether routine or standard oedding disposal procedures are acceptable

☐ [illegible]

☐ [illegible]

12.4.1

12.4.2

Other Bedding Disposal Procedures 12.4.2.1

Descnbe the non-routine or special bedding disposal procedures

---

Special Carcass Removal Procedures 12.5

Indicate whether routine or standard carcass removal procedures are acceptaole

☐ [illegible]

☐ [illegible]

12.5.1

12.5.2

Description of Special Carcass Removal Procedures 12.5.2.1

Descnbe the non-routine or special precautions for carcass removal

---

Special Decontamination Procedures 12.6

Indicate whether routine or standard decontamination procedures are acceptable

☐ [illegible]

☐ [illegible]

12.6.1

12.6.2

Description of Special Decontamination Procedures 12.6.2.1

Descnbe non-routine or special precautions fordecontamination procedures

---

Investigator Assurances & Compliance Agreement

(b)(6)

Principal Investigator Assurances and Compliance Agreement

13.1

I will conduct this study in accordance with all applicable rules and regulations as described in the PHS Policy on Humane Care and Use of Laboratory Animals, the Federal Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, and CDC/NIOSH Morgantown internal policies. (Click the Help icon for links to these resources.)

I will obtain approval from the IACUC before initiating any change in the study objectives, design, or procedures. I understand that work performed without IACUC approval cannot be published with certification of IACUC approval and may result in federally-required reporting of noncompliance.

I have determined that the research proposed is not unnecessarily duplicative.

I have reviewed the pertinent scientific literature and the sources and/or databases noted in this proposal and found no scientifically acceptable alternatives to any procedures that may cause pain or distress.

I confirm that all individuals working on this protocol are enrolled in NIOSH's Occupational Health and Safety Program.

I authorize individuals listed on this protocol to conduct procedures involving animals, and I accept responsibility for their oversight in the conduct of this proposal. I certify that these same individuals will read, understand, and follow the procedures in the final approved version of this protocol and any future amendments.

I confirm that all individuals listed on this protocol have obtained authorization to work with animals in this facility by the Animal Facilities Director or will be required to do so before work with animals begins. Further, I certify that these individuals are properly trained, or will receive such training prior to work with animals, in all areas relevant to their assigned work with animals (e.g., biology, handling, and care of the species used; aseptic surgical methods and techniques; the concept of 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; and procedures for reporting animal welfare concerns.)

I certify that all individuals listed on this protocol will complete any additional training, continuing education, or refresher training as assigned by the IACUC.

I understand that I am responsible for assuring that my laboratory is in compliance with all federal, state, and local environmental laws and regulations. I accept the responsibility to assure that my laboratory is operated in a safe manner and that all staff and students are informed of potential risks, wear appropriate personal protective equipment, and are adequately trained. I will assure that all personnel working on this protocol receive orientation to laboratory safety instructions, SDS files, and laboratory emergency procedures. I am responsible for adhering to NIOSH safety policies and procedures for handling hazardous materials and for addressing accidental spills and personnel contamination. I will report any significant problems, and research-related accidents, and illnesses to the Safety Office, and will complete the required forms in the event of an incident.

For animals under this protocol, I accept that in the case of necessary veterinary treatment, the Attending Veterinarian is authorized to provide treatment required to sustain life or, if necessary, provide humane euthanasia to prevent unapproved pain and/or distress. I understand that the animal facility staff will contact me as soon as possible using the emergency contact information that I provide in this protocol, but I understand that such contact may not always be possible prior to treatment or performing euthanasia.

I will notify the IACUC of unanticipated adverse outcomes. Unanticipated outcomes are generally defined as negative impacts to animal welfare or well-being that are not explicitly described in the protocol and approved by the IACUC. These include unanticipated outcomes associated with disease, injury, or effects that are under investigation.

I accept that veterinary consultation must occur when pain or distress is beyond the level the IACUC approved in the protocol, or when animal facility staff are unable to provide interventional control (i.e., immediate euthanasia). I will notify the Attending Veterinarian when unanticipated pain or distress, unexpected morbidity, or unanticipated mortality occurs. This applies to animals housed/used at NIOSH and animals funded by NIOSH and housed/used at collaborating institutions.

☐ Agree

13.1.1

(b)(6)

ATTACHMENT Section 13.2

Use this section to attach any documents used to illustrate what you have written in the sections above Keep in mind that attachments are not printed out with the protocol and that the information provided in the protocol must stand on its own
The IACUC prefers files in pdf images. WORD and Excel files should be saved as pdf before attachment.
Attach the file using the paperclip icon at the right
For each file attached complete the description below

Date Document Added	File Name	File Content/Description	Protocol Sections

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For IACUC Administration Use Only

IACUC Admin Section 14.1

This section will be used for the IA to add correspondence or other administrative documents

☐ [illegible]

☐ N...

14.1.1

14.1.2

Comments 14.2

Appendix 9 4 IACUC Protocol Form- Annual Renewal for Expired Protocols

CDC- Morg IACUC - Fiscal Year 2019 Annual Report on Animal Use and Protocol Update

(Use this form only if your IACUC protocol expired in FY2019 (i.e. expired prior to October 1, 2019)

PI Name (Print):_____ Protoco Number: _____

1. Protcol Status during the reporting period of October 1, 2018– September 30, 2019 (**Choose one**)

   ☐ 1a Protocol was active during FY19 (i.e animals were used during this reporting period).

   ☐ 1b Protocol was nactive during FY19 (i.e. no anima s were used, on site or received on this protoco during this reporting period)

   If you answered "b", then go to end of form, sign and date, you have completed the annual report for this protocol for FY19.

2. Animal Use during the reporting period (**Complete thefollowing:**)

Pain Category	Species	Carryover from FY18	Vendor Sourced in FY19	NBP Sourced in FY19	Carryover to FY20

- Pain category would be E, D or C;
- Carryover from FY18 is animals that were on site under your protocol on September 30, 2018 that were still on site the following day October 1, 2018;
- Vendor sourced in FY19 were any animals you received from a commercial vendor that were received on October 1, 2018 through September 30, 2019;
- NBP Sourced in FY19 were any animals received from the NBS Breeding Program on October 1, 2018 through September 30, 2019;
- Carryover to FY20 are animals that were on site under this protocol on September 30, 2019 that were still on site the following day October 1, 2019.

3. Animal Disposition during the reporting per od (**Complete the following:**)

Species	Euthanized before study endpoint	Died before study endpoint	Euthanized at study endpoint	Transferred to another protocol

4. Special Activities during the reporting period (**Choose all that apply**)

- ☐ Study animals were single housed for scientific reasons as described in the approved protocol.
- ☐ Study animals were prevented from having enrichment as described in the approved protocol.
- ☐ Study animals were housed in less than the minimum regulated space.
- ☐ Study animals were housed in a Satellite Animal Housing Facility
- ☐ Animals were held in a laboratory (i.e. outside of the Animal Facility) for more than 12 hours.
- ☐ Study animals were housed under environmental conditions outside of the recommended ranges.
- ☐ Animals underwent survival surgical procedures.
- ☐ Animals underwent non-survival surgical procedures (does not include euthanasia solution followed by terminal perfusion).
- ☐ Animals experienced pain/distress that was more than momentary and could not for scientific reasons be treated with anesthetics or analgesics (i.e. category E procedures/experimental agent).
- ☐ None of these conditions apply.

5. NIOSH Breeding Program Use (Choose **one** and complete table as appropriate.)

- ☐ N/A – I am not approved to use animals from the NBP for experiments.
- ☐ I am approved to use animals from NBP on this protocol but did not request or use any during this reporting period.
- ☐ I am approved to use animals from the NBP on this protocol for experiments and have summarized usage below

Strain Code	Sex	Number Requested in Reporting Year	Number Used in Reporting Year	Planned Use in FY20

6. Unexpected Adverse Events

- ☐ No unexpected adverse events occurred during this reporting period
- ☐ Yes, unexpected adverse events occurred during this reporting period as described in the attachments.

Attach to this report the date(s) of each event, persons involved, persons present, the number of animals effected by the event and any actions taken to minimize the effects. Document if you contacted the AV or IACUC of this event. Include in this report a summary of the corrective actions taken to minimize the effects or prevent recurrence of each event.

ATTACHMENTS

7 Future Plans (**choose one**)

- ☐ Exper ments as part ofth s study have been completed or have beer terrrinated
- ☐ A renewal to continue these studies has been submitted.
- ☐ A renewal to continue these studies will kely be subm tted in the future

8. Ongoing studies - In the space provided below, briefly summarize what animial work is planned for Fiscal Year 2020

Assurances– by signing th s form you also agree and accept the following assurance statements:

- A l protocol assoc ates are trained and proficient in the animal procedures descr bed
- A l protocol assoc ates have documented their tra ning records during the reporting period
- A l protocol associates working with anima s have reviewed and have access to my currently approved protocol.
- All protocol associates understand that any modifications (additions or changes) to this protocol requ re ACUC review and approval.
- have accepted the 3Rs when appropriate to rrinimize pain anddistress.
- have reviewed the literature and this research is not unnecessarilydup icative.

PI Signature

Date

PRINT SUBMIT

Appendix 10: Semi-Annual Report

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control and Prevention National Institute for Occupational Safety and Health

Memorandum

Date: November 6, 2019

From: NIOSH/HELD Animal Care and Use Committee

Subject: Semiannual Program Review and Facilities Inspection Report (May 2019– Oct 2019)

To: Steve Monroe, Ph.D., Associate Director for Laboratory Science and Safety and IACUC Institutional Official (CDC/OD/OADLSS)
Through: Don Beezhold, Ph.D., Director, CDC/NIOSH/HELD____

This is the semiannual report for the NIOSH (CDC-Morgantown) Institutional Animal Care and Use Committee (IACUC) for the period of 1 May 2019– 31 October 2019 as required by the PHS Policy on Humane Care and Use of Laboratory Animals and as a condition of this institution's Animal Welfare Assurance D16-00687 (Old Assurance# A4367-01) on file with the Office of Laboratory Animal Welfare (OLAW) and USDA Animal Welfare Regulations, 9 CFR Chapter I, subchapter A, as applicable (NIOSH is covered under the CDC-Atlanta USDA Registration #57-F-0004). The ACUC utilizes the Guide for Care and Use of Laboratory Animals (Guide), 8th edition, the Animal Welfare Regulations (9 CFR Chapter I) and OLAW policies as the basis for program evaluation.

Animal Welfare Assurance for Domestic Institutions Renewal

The PHS Assurance Renewal for CDC-Morgantown Program D16-00687 (old A4367-01) was submitted to OLAW, Division of Assurances on 30 August 2019.

AAALAC Accreditation Status

Full Accreditation Status. Program Description will be due December 1, 2019, with a site visit scheduled the Winter Trimester (Jan - Mar 2020).

Programmatic Changes since last report

- New policies drafted and approved by the IACUC:
    - CDC-Morgantown IACUC Policy P-007 on the Reporting and Investigation of Animal Welfare Concerns. Approved 16 May 2019.
    - CDC-Morgantown IACUC Policy P-008 on Post-Approval Monitoring. Approved 19 September 2019.

- CDC-Morgantown IACUC Policy P-009 on USDA Pain Categories. Approved 24 October 2019.

ACUC Roster Changes

No roster changes during this semi-annual program review and inspection period.

USDA Covered Species – None were housed during this period.

ACUC Approved Departures from the Guide

Animal Protocol (b)(7)(E) Non-standard Housing) The studies require short term (up to 3 days) housing of rats in wire floored rat metabolism cages in 12-hour intervals to collect urine. Animals have sufficient space to stand up, turn around and lie down. Food and water are still available ad libitum and the ACUC has approved for a minimum time period. Rats are housed in bedded cages of appropriate size when not in the metabolism cage.

Animal Protocol (b)(7)(E) Use of Prolonged Restraint). Mouse and rat nose-only inhalation studies utilize rigid tubes for whole body prolonged restraint. Animals are acclimated to tubes prior to experiment and exposures/restraint time is limited to only what is scientifically necessary to provide exposure (Usually less than 2 hours).

Animal Protocol (b)(7)(E) Use of Prolonged Restraint). Rats will be placed in Broome restrainers for prolonged whole-body restraint for exposure and physiological testing. The animals are acclimated to the Broome restrainers prior to the experiments (Usually less than 1 hour).

Confinement versus Restraint

There are many mice and rat inhalation studies where animals are dosed with particles via whole body exposure. The animals are confined but are able to turn around and lie down in wire cage batteries. The animals frequently sleep during the procedure and rarely see lesions due to the wire. Exposures may last anywhere from 1-6 hours per day on multiple days. The ACUC has determined that this is not restraint.

Program Review

The Semiannual Program Review was conducted during the ACUC meeting October 24, 2019 with a quorum present. The ACUC utilized the OLAW checklist to perform program review. As part of the Semiannual Program Review, the IACUC reviewed the current ACUC Policies: 1) CDC-Morgantown IACUC Policy P-001 on the Social Housing of Mice and Rats, 2) CDC-Morgantown IACUC Policy P-003 on Euthanasia, 3) CDC-Morgantown IACUC Policy P-004 on Conflict of Interest, 5) CDC-Morgantown IACUC Policy P-005 on the Satellite Animal Facility and 6) CDC-Morgantown IACUC Policy P-006 on the Review of Protocols and Modifications to Protocols. P-003 and P-005 are under revision to improve and describe current IACUC/ ACUP procedures.

Appendix 10: Semi-Annual Report

Facility Inspections

The findings from the Semiannual Inspections of the Animal Facility and laboratories where animals are used were discussed and categorized at a convened meeting on October 24, 2019. Updates to the Animal Welfare posters were not required as all information remained current. The ACUC provided "Expired- Not for use in animals" stickers during the inspection to make on the spot corrections during the inspection. The inspection forms for the laboratories and animal facility were revised to capture the animal activities performed in laboratories and specific room types (breeding rooms, animal housing rooms, inhalation rooms, cage washing, etc.) in the animal facility. The inspection forms for the laboratories and animal facility were sent to the investigators, Attending Veterinarian and Animal Facility Manager one week in advance of the scheduled inspections and requested that the forms be filled out and returned to the IACUC prior to the lab inspection. The IACUC inspection team reviewed the forms prior to visiting the labs and followed up on the information provided by the investigators. A Division (HELD)SOP (SOP-OD-00346) for the handling and use of controlled substances (Schedule I and II) was developed as a guidance on division (and DEA) requirements. The IACUC inspection team discussed/ confirmed that PIs were aware of the SOP during the inspection.

The Fall Semi-annual Inspections were conducted on the following dates –

Animal Facilities – October 3, 2019.

Investigator laboratories where animals are used – October 7 – 9, 2019.

Significant Deficiency

No significant deficiencies were identified during this semi-annual program review and inspection period.

Minor Deficiency

Animal Facility –

- One bottle of expired antibiotic found in animal facility pharmacy cabinet and one bottle found in one animal housing room were removed from animal facility and the deficiency corrected, 3 October 2019.
- Expired disinfectant removed/ replaced in several rooms. The deficiency was corrected, 7 October 2019.
- Monthly eyewash inspection logs out of date in several rooms. This deficiency was corrected by the Animal Facility technicians, 21 October 2019.

Research Laboratories –

- The most common deficiency found in several laboratories was that the ACUC notebooks were not up to date and did not contain all current protocols and the Animal Emergency Response Plan Appendix 3 did not have the signatures of all the protocol associates identified in the protocols in the book. The deficiencies in recordkeeping were corrected by the PIs and confirmed by the IACUC Administrator by 17 October 2019.
- One lab stored the key to the (b)(7)(E) the key was moved to a concealed location. The deficiency was corrected, 8 October 2019.

Reports to OLAW

OLAW Case (b)(7)(E) human error in administration of agent to animals, 28 June 2019.

OLAW Case (b)(7)(E) improper euthanasia method to ensure death of animal, 16 September 20019

Cage containing mice discovered without water bottle- reported to IO, 10 September 2019; IO sent report to OLAW, 2 October 2019 (Case number to be assigned).

Minority Views

There are no minority views to report.

Signatures

Signatures of the ACUC members are provided below.

Names of Voting IACUC Members	Signatures
(b)(6)	

Semiannual Report for May 2019 – October 2019

cc: ACUC file
Don Beezhold
Steve Monroe
Linda Pimentel, ACUPO Office
(b)(6)

Appendix 11.2 Heating, Ventilation and Air Conditioning (HVAC) System Summary

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

Location/Building/Facility:	**Animal & Inhalation Facility**	(b)(7)(E)

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.

This is a 100% outdoor air system utilizing a central chilled water plant, steam plant, clean steam humidification plant and water reheat system. All are controlled by a central building automation system maintaining a set supply air static pressure at a constant 55 deg. Each room maintains air flow utilizing Phoenix control system air volume control valves maintaining a set rate of air changes. Room temperature is maintained by a reheat in each room. Air humidity is maintained by a central humidifier in the air unit and a local booster for the AF area. To maintain air quality the system uses a 40% pre- filter, 95% final filter, and HEPA filtration with booster fan. This entire system is monitored by the central building control system (Siemens) 24/7, 365 days and will alert by paging the Facilities Maintenance Office (FMO) on-call person and also the building security guard if any portion of the system has a malfunction including static pressure, system power loss, system shutdown, high or low discharge temp, loss of steam pressure, and out of range relative humidity (RH). The building control system utilizes stand-alone room controllers and will continue to operate if connection is lost to the central system. All room controllers are connected to the backup power system and will continue to operate during a power outage. The central HVAC system is connected to the backup power system and will operate at a reduced rate during a power outage; it will maintain heating and humidification but will not maintain cooling. The humidification system utilizes a high limit shutdown. A new backup chiller was installed for the AF core part of the facility in the spring of 2018 to provide cooling redundancy should the chilled water system fail. This backup chiller does not support the inhalation corridor, so in the event of system failure the animals in this area would need to be moved to

the core area until the system is returned to working order. In the past three years our aging environmental controls system has struggled to maintain temperature and humidity, with overnight and weekend excursions becoming common, and replacement parts for faulty control valves and sensors are not always available. Recently we've experienced unintended changes in animal room differential pressure. Replacement and modernization of the environmental controls for the core AF and inhalation corridor in (b)(7)(E) are planned with a FY2021 priority funding request submitted through NIOSH to the CDC.

(b)(7)(E) – This room is serviced by a different air handler; however, all the animals are housed in IVC. An updated building management system for the (b)(7)(E) (Distech Controls) now monitors temperature, RH, % reheat and air changes per hour, and adjustments can be made centrally. The major drawback to this room is that humidity cannot be added to the air handler supplying this room.

Appendix 11.2 Heating, Ventilation and Air Conditioning (HVAC) System Summary

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

Room No.	**Specific Use**	**Temperature Set-Point** (define units)	**Electronic / Emergency Monitoring of Temperatures** (Y/N)	**Alert/Alarm Temperature Ranges** (all in Fahrenheit)	**Humidity Control** (Y/N)	**Relative Pressure**	**Air Exchange Rate** (per hour)	**Date Verified / Measured**
		(settings verified)					**(Supply values measured)**	
(b)(7)(E)	Clean Storage	70°F	Y	68/77	Y	+	not measured	
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	+	10	5/2020
	Animal Housing/Study Room- Rats	72°F	Y	68/75	Y	+	18	5/2020
	Technician Office	70.5°F	Y	65/75	Y	-	not measured	
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	+	16	5/2020
	Animal Housing/Study Room – Mice	72°F	Y	65/76	Y	+	20	5/2020
	Animal Housing Room – Mouse Breeding	71°F	Y	68/75	Y	+	11	5/2020
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	+	12	5/2020
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	+	13	5/2020
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	+	13	5/2020

Appendix 11.2 Heating, Ventilation and Air Conditioning (HVAC) System Summary

Room No.	Specific Use	Temperature Set-Point (define units)	Electronic / Emergency Monitoring of Temperatures (Y/N)	Alert/Alarm Temperature Ranges (all in Fahrenheit)	Humidity Control (Y/N)	Relative Pressure	Air Exchange Rate (per hour)	Date Verified / Measured
		(settings verified)					(Supply values measured)	
(b)(7)(E)	Animal Housing/Study Room- Mice	72°F	Y	68/75	Y	+	16	5/2020
	Animal Housing/Study Room- Mice	72°F	Y	68/75	Y	+	16	5/2020
	Animal Housing/Study Room- Rats	72°F	Y	68/75	Y	-	5	5/2020
	Animal Housing Room – Mouse Breeding	72°F	Y	68/75	Y	-	8	5/2020
	Surgical/ Multipurpose Procedures Room	72°F	Y	68/75	Y	+	not measured	
	Animal Housing/Study Room – Rats	72°F	Y	68/75	Y	-	14	5/2020
	Euthanasia & Necropsy	72°F	Y	68/75	Y	-	not measured	
	Animal Housing Room – Inhalation Rats	72°F	Y	68/79	Y	-	13	5/2020
	Animal Housing Room – Inhalation Mice	72°F	Y	68/79	Y	-	17	5/2020
	Satellite Animal Housing – Mice and Rats	72°F	Y	68/75	N	-	8	5/2020

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

Copy and repeat the Description and Table for each location, including all sat

Appendix 13.1: Primary Enclosures and Animal Space Provisions

Please complete the Table below considering performance criteria and guiding documents (e.g., Guide, Ag Guide, ETS 123 and/or other applicable standards) used by the IACUC/OB to establish adequacy of space provided for all research animals including traditional laboratory species, agricultural animals, aquatic species, and wildlife when reviewing biomedical, field, and agricultural research studies.

Species	Dimensions of Enclosure (cage, pen, tank*, corral, paddock, etc.)	Maximum Number Animals / Enclosur	Guiding Document Used to determine the Institution's Space Standards (Guide, Ag Guide, Other)	Enclosure Composition & Description**
Mouse	Super Mouse Cage (96 in^2) 12.8x7.5x5 in	5, > 26 grams	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Mouse	NexGen Mouse Cage (77.5 in^2) 7.6x7.1x15.7 in	5, > 26 grams	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Mouse	Bluel284L (82 in^2) 12.7x6.4x5.3 in	5, > 26 grams	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Mouse	Duplex cage (51 in^2) 12.1x5.5 in	2, > 26 grams	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Mouse	Weaning (112 in^2) 12x12x5.5 in	6, > 26 grams	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Rat & Guinea Pig	One Cage (210 in^2) 12.3x17.2x7.2 in	3 F344 > 500g 2 SD > 500g 2 GP > 351g	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Rat	Thoren Rat cage (115 in^2) 12x12x7.5 in	1, > 500g	Guide	Solid bottom, IVCS, polycarbonate shoebox type
Rat	Nalgene #650-0350 7.125 in tall x 9.25 diameter (70 in^2)	1	Guide	Wire bottom, static, polycarbonate, metabolism cage

*For aquatic species, provide tank volume.

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

Appendix 13.2: Primary Enclosures and Animal Space Provisions
Animal Density Chart

MICE

Lab Products Super Mouse Cage

96 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	10	6	12
11	15	8	9
16	25	12	6
26+		15	5
Female w/ Litter		51	1

Dimensions > 12.8 x 7.5 x 5 inches

Thoren Duplex Cage

51 7 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	10	6	8
11	15	8	6
16	25	12	3
26+		15	2
Female w/ Litter		51	X

Dimensions > 12.1 x 5 x 5 inches

Allentown NexGen Standard Cage

77.5 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	10	6	12
11	15	8	9
16	25	12	6
26+		15	5
Female w/ Litter		51	1

Dimensions: 7.625 x 7.11 x 15.68 inches

Thoren Weaning Cage

112.9 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	10	6	12
11	15	8	10
16	25	12	8
26+		15	6
Female w/ Litter		51	1

Dimensions > 12.1 x 12.1 x 5.5 inches

Tecniplast Blue Line, 1284L

82 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	10	6	12
11	15	8	9
16	25	12	6
26+		15	5
Female w/ Litter		51	1

Dimensions: 12.71 x 6.46 x 5.3 inches

Appendix 13.2: Primary Enclosures and Animal Space Provisions
Animal Density Chart

RATS

[redacted]

Lab Products One Cage 2100 Rat Cage

210 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage		Cage w/ Divider
0	100	17	12		5
101	200	23	9		4
201	300	29	7		3
301	400	40	5		2
401	500	60	3		1
501	F344	70	3		1
	SD		2		1
Female w/ Litter		124	1		X

Dimensions > 12.3x17.2x7.2 inches

[redacted]

Thoren Rat Cage

115.1 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage
0	100	17	6
101	200	23	5
201	300	29	4
301	400	40	2
401	500	60	2
501	F344	70	1
	SD		1
Female w/ Litter		124	X

Dimensions 12.1x12.1x7.5 inches

GUINEA PIG

[redacted]

Lab Products One Cage 2100 Cage

210 sq in floor space

Weight Range (grams)		Min Sq in	Whole Cage	Calc	Cage w/ Divider
0	350	60	3		1
351		101	2		x

Dimensions > 12.3x17.2x7.2 inches

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

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

Area	**Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)**	**Washing/ Sanitizing Frequency**	**Chemical(s) Used***	**Other Comments (e.g., autoclaved)**
Micro-environment				
Solid-bottom cages (static)	Mechanical washer	1x/ week	Citric acid detergent	Rarely used
Solid-bottom cages (IVC)	Mechanical washer	1x/ 2 weeks minimum	Citric acid detergent	Some autoclaved; longer interval for low density cages
Suspended wire-bottom or slotted floor cages – Rodent metabolism cages	Mechanical washer	Weekly	Citric acid detergent	
Cage lids (aka 'steel') Filter tops Feed jars	Mechanical washer	At least monthly (every other cage change)	Citric acid detergent	Autoclaved after washing
Cage racks and shelves	Hand sanitized during regular cage change; Mechanical washer annually	Every cage change for hand sanitizing; annually for mechanical	Quaternary PV, Virkon; Rescue or Clorox H2O2 in designated "Quat-free" rooms Citric acid detergent annually	Surfaces are wiped with disinfectant at each cage change.
Watering bottles	Mechanical washer	Each cage change (every 2 weeks)	Citric acid detergent	Autoclaved after washing

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

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Exercise devices and manipulanda used in environmental enrichment programs, etc.	Mechanical washer	Every other cage change (at least monthly)	Citric acid detergent	Autoclaved after washing
Transport carts	Hand wash	After use/ daily	QAC	Wheels wiped with QAC during transport through airlocks
Dunnage racks	Hand wash	Every 6 months	QAC	In feed and bedding storage
Pallets	Mechanical washer	Every 6 months	Citric acid detergent	
Operant conditioning & recording chambers, mechanical restraint devices (chairs, slings, etc.)	N/A			
Euthanasia chambers	Hand wash	After use	QAC	Rodent home cage preferred, otherwise use a clean shoebox cage
Supply Carts in Animal Rooms	Hand wash	Every 6 months	QAC	
Macro-Environment				
Animal Housing Rooms: AF Core, Inhalation and (b)(7)(E)				
Floors	Squeegee-swept, sprayed with chemical and mopped	Daily/weekly	QAC (peroxymonosulfate for BSL2)	

Walls	Mopped	Monthly	QAC (peroxymonosulfate for BSL2)	

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Ceilings	Mopped	Monthly	QAC (peroxymonosulfate for BSL2)	
Ducts/Pipes				Not exposed
Fixtures	Wiped with chemicals	Daily	QAC (peroxymonosulfate in BSL2) followed by 70% alcohol	
Corridors:				
Floors	Mopped	Daily	QAC	
Walls	Mopped	Every 6 months or more often if needed	QAC	Windows every 6 months
Ceilings	Mopped	As needed	QAC	
Ducts/Pipes				Not exposed
Fixtures	Hand sanitized	As needed	QAC	
Animal Room turnaround	Fogging	As needed	Chlorine dioxide or peroxide vapor	When rooms go down or for decontamination
Support Areas (e.g., surgery, procedure rooms, etc.); complete for each area:				
Floors	Squeegee-swept, sprayed with chemical and mopped.	Daily / weekly	QAC	Depends on use. If not in use, is spot checked weekly and mopped
Walls	Hand sanitized with mop	As needed	QAC	
Ceilings	Hand sanitized with mop	As needed	QAC	

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

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/ Sanitizing Frequency	Chemical(s) Used*	Other Comments (e.g., autoclaved)
Ducts/Pipes				Not exposed
Fixtures	Hand sanitized	As needed	QAC	
Room turnaround	Fogging	As needed	Chlorine dioxide or peroxide vapor	When room function changes to animal room or for decontamination
Implements (note whether or not shared):				
Mops	Rinsed and Autoclaved	After use	QAC	Autoclaved. Not shared and replaced frequently
Mop buckets	Hand washing	Every 4-6 weeks	QAC	
Other				
Other:				
Vehicle(s)	Not applicable			

*Please provide chemical, not trade name.

Appendix 15: Facilities and Equipment for Sanitizing Materials

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

[*Note*: Please remove the examples provided in the Table below.]

Building	Room No.	Equipment Type	Safety Feature(s)	Methods of Monitoring Effectiveness
(b)(7)(E)		Tunnel Washer	Emergency "off" button; instructional signage	Guarantee 180F hot water rinse verified on output; temperature sensitive tape used with every load
		Rack Washer	Emergency "off" button; labeled exit door; de-energizing cord on both sides; instructional signage	Guarantee 180F hot water rinse verified on output; temperature sensitive tape used with every load
		Bulk Autoclave	Emergency 'off' button	Temperature sensitive tape each load and Diack Sterilization Monitor weekly.

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

Appendix 16: Lighting Summary

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

Location:	**AF Core** (b)(7)(E) **+ SAF room**

Room Type[a]	**Light Intensity Range**	**Lighting Fixture Construction Features**[b]	**Photo-period** (hrs)[c]	**Photoperiod and Lighting Control**	**Override Mechanisms** (if applicable)
Rodent Holding Rooms	20-86 (Avg. 39) foot candles	Surface mounted, water resistant	12:12	Automatic via building management system	Yes, 15 minute manual override
Rodent Breeding Rooms	20-66 (Avg. 33) foot candles	Surface mounted, water resistant	14:10	Automatic via wall-mounted timer box	Yes, 15 minute manual override
Surgery, (b)(7)(E)	80-110 foot candles	Surface mounted, water resistant	12:12	Automatic via wall-mounted timer box	Yes, 15 minute manual override
AF Procedure Room (Necropsy, (b)(7)(E)	100-110 fc (10-40 fc inside the Euthanex system)	Recessed, water resistant; arm-mounted, water resistant	12:12	Automatic via wall-mounted timer box	Yes, 15 minute manual override
Cage-Washing Room	60-90 fc	Recessed, water proof	NA	Manual Switch	N/A
(b)(7)(E)	27-37 foot candles	Surface mounted; water resistant	12:12	Automatic via building management system	N/A

[Measures are being taken to mitigate light intensity in some of the animal holding rooms.]

[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.

Appendix 17: Satellite Housing Facilities

Note: In the Program Description Section 2. IV. (Physical Plant), item C., describe the criteria used to determine a "Satellite Animal Holding Area." In the Table below, summarize these animal housing areas. Note that each of these must also be included in the Heating, Ventilation, and Air Conditioning (HVAC) Summary (**Appendix 11**) and Lighting Systems Summary (**Appendix 16**).

Building	Room(s)	Person Responsible	Species Used	Approximate Area (ft^2 or m^2) Devoted to Housing	Maximum Period of Stay	Purpose / Rationale / Justification	Construction Features and Finishes
(b)(7)(E)		AF Director & AF Supervisor	Rats and mice	240 sq. ft.	No limit	For experimental animals that require back & forth movement between housing and PI labs in the (b)(7)(E) Quarantine- in the event it is needed.	Concrete epoxy coved flooring, laminated walls over drywall

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

Appendix 18: Animal/Inhalation Facility Contractors Personnel Training Summary

Role	Degrees	Certifications	AALAS Mtg	TRBAALAS Member	Yrs @ NIOSH
Animal Facility Supervisor	BS, Animal & Veterinary Science	CMAR, RLATG + ILAM graduate (2011)	9	Y	21.5
ACT	AS	RALAT	1	Y	2
ACT with Breeding Colony Responsibilities	AS, Veterinary Technology	RALAT	1	Y	3
ACT	high school + PCDI correspondence course	RLAT	4	Y	21
ACT	AS	RLAT	3	Y	9.5
ACT	high school	RALAT	3	N	13.5
ACT	BS, An. Sci & Nutrition		1	Y	0.5
ACT	BS, Biology	RALAT	N	N	1.5
		YEARS>	22		72
Inhalation Tech	BS	LAT	1	N	15
Inhalation Tech	BS		N	N	10
Inhalation Tech	BS		N	N	5.5
Inhalation Tech	BS		N	N	0.5
		YEARS>			31

Appendix 19: Rodent Health Surveillance

Rat Serology Profiles

	Primary	Clinical	Basic	Compreh	Global
RCV/SDAV	•	•	•	•	•
NS1 (Generic Parvovirus)	•	•	•	•	•
RPV	•	•	•	•	•
RMV	•	•	•	•	•
KRV	•	•	•	•	•
H-1	•	•	•	•	•
RTV (Rat theilovirus)	•	•	•	•	•
Pneumocystis carinii	•	•	•	•	•
Sendai virus		•	•	•	•
PVM		•	•	•	•
Mycoplasma pulmonis		•	•	•	•
REO3			•	•	•
LCMV			•	•	•
CARB				•	•
Hantaan virus				•	•
Clostridium piliforme				•	•
MAV1				•	•
MAV2				•	•
Encephalitozoon cuniculi					•
IDIR					•
Rat Polyomavirus 2 (RPyV2)					•

Mouse Serology Profiles

	Primary	Clinical	Basic	Compreh	Comprehe	Global
MHV	•	•	•	•	•	•
MVM (MMV)	•	•	•	•	•	•
NS1 (Generic Parvovirus)	•	•	•	•	•	•
MPV 1-5	•	•	•	•	•	•
MNV	•	•	•	•	•	•
TMEV	•	•	•	•	•	•
EDIM	•	•	•	•	•	•
Sendai virus		•	•	•	•	•
Mycoplasma pulmonis		•	•	•	•	•
PVM			•	•	•	•
REO3			•	•	•	•
LCMV			•	•	•	•
Ectromelia virus			•	•	•	•
MAV1				•	•	•
MAV2				•	•	•
Polyomavirus				•	•	•
Encephalitozoon cuniculi					•	•
CARB					•	•
Clostridium piliforme					•	•
MCMV					•	•
K virus						•
Hantaan virus						•
LDV						•
MTV						•

Mouse Opti-XXPress PCR Profiles

	Prevalent	Basic	Comprehensive	Global	SampleType*
Bacteria - Respiratory					
Pasteurella pneumotropica (Jawetz and Heyl biotypes)	•	•	•	•	F, OS, E
Mycoplosmo pulmonis		•	•	•	F, OS, E
Staphylococcus aureus			•	•	F, OS, E
Streptococcus, β-hemolytic (Groups A, B, C, G)			•	•	F, OS, E
Bordetello bronchiseptica				•	F, OS, E
Bordetella hinzii				•	F, OS, E
Corynebocterium kutscheri				•	F, OS, E
Streptobocillus moniliformis				•	F, OS, E
Streptococcus pneumonioe				•	F, OS, E
CARB				•	F, OS, E
Bacteria - Enteric					
Helicobacter spp. – *H. bilis, H. ganmani, H. hepaticus, H. mastomyrinus, H. rodentium, H. typhlonius*	•	•	•	•	F, E
Klebsiella oxytoca		•	•	•	F, E
Klebsiello pneumoniae		•	•	•	F, E
Solmonello spp.		•	•	•	F, E
Citrobacter rodentium			•	•	F, E
Clostridium piliforme			•	•	F, E
Pseudomonas aeruginosa			•	•	F, E
Campylobacter spp.				•	F, E
Proteus mirabilis				•	F, E
Bacteria - Skin					
Corynebocterium bovis				•	FS, F, OS, E
Staphylococcus xylosus				•	FS, F, OS, E
Fungi					
Pneumocystis spp.				•	L, E
Viruses					
MHV	•	•	•	•	F, E
MVM	•	•	•	•	F, E

MPV (1-5)	•	•	•	•	F, E
MNV	•	•	•	•	F, E
TMEV	•	•	•	•	F, E
MRV/EDIM	•	•	•	•	F, E
MAV1, MAV2			•	•	F, E
Mouse Kidney Parvovirus (MKPV) **NEW!**				•	F, E
Reo virus 1, 2 and 3				•	F, E
PVM				•	F, E
Sendai virus				•	F, E
Ectromelia				•	F, E
Hantaan virus				•	F, E
Lymphocytic choriomeningitis virus				•	F, E

Rat Opti-XXPress PCR Profiles

	Prevalent	Basic	Comprehensive	Global	Sample Type*
Bacteria - Respiratory					
Pasteurella pneumotropica (Jawetz and Heyl biotypes)	•	•	•	•	F, OS, E
Mycoplasma pulmonis		•	•	•	F, OS, E
Staphylococcus aureus			•	•	F, OS, E
Streptococcus, β-hemolytic (Groups A, B, C, G)			•	•	F, OS, E
Bordetella bronchiseptica				•	F, OS, E
Corynebocterium kutscheri				•	F, OS, E
Streptobocillus moniliformis				•	F, OS, E
Streptococcus pneumonioe				•	F, OS, E
CARB				•	F, OS, E
Bacteria - Enteric					
Helicobocter spp. –*H. bilis, H. ganmani, H. hepoticus, H. mastomyrinus, H. rodentium, H. typhlonius*	•	•	•	•	F, E
Klebsiella oxytoca		•	•	•	F, E
Klebsiella pneumoniae		•	•	•	F, E
Salmonella spp.		•	•	•	F, E

Clostridium piliforme			•	•	F, E
Pseudomonas aeruginosa			•	•	F, E
Campylobacter spp.				•	F, E
Proteus mirabilis				•	F, E
Bacteria - Skin					
Staphylococcus xylosus				•	FS, F, OS, E
Fungi					
Pneumocystis spp.				•	L, E
Viruses					
RCV/SDAV	•	•	•	•	F, E
RPV	•	•	•	•	F, E
KRV	•	•	•	•	F, E
H-1	•	•	•	•	F, E
RMV	•	•	•	•	F, E
RTV	•	•	•	•	F, E
BCV			•	•	F, E
MAV1, MAV2			•	•	F, E
Reo virus 1, 2 and 3				•	F, E
PVM				•	F, E
Sendai virus				•	F, E
Seoul virus				•	F, E

Pinworm, Fur mite and Protozoal PCR Profile

Fur Mite PCR Profile • *Myocoptes* • *Myobia* • *Radfordia*
Pinworm PCR Profile for Mice • *Syphacia obvelata* • *Syphacia muris* • *Aspiculuris tetraptera*
Pinworm PCR Profile for Rats • *Syphacia muris* • *Syphacia obvelata* • *Aspiculuris tetraptera*
Pinworms and Fur Mites PCR Profile For mice or rats Includes species of pinworms and fur mites listed above.
Protozoal PCR Profile • *Entamoeba muris*

- *Giardia muris*
- *Spironucleus muris*
- *Tritrichomonas muris*

***Helicobacter* spp.**

Helicobacter bilis

Helicobacter ganmani

Helicobacter hepaticus

Helicobacter mastomyrinus

Helicobacter rodentium

Helicobacter typhlonius

IMPACT Mouse Profiles

	IMPACT I	IMPACT II	IMPACT III	IMPACT IV	IMPACT SC
Mycoplasma spp.	•	•	•	•	•
Mycoplasma pulmonis	•	•	•	•	•
Mouse hepatitis virus (MHV)	•	•	•	•	•
Minute virus of mice (MVM)	•	•	•	•	•
Mouse parvovirus (MPV1-5)	•	•	•	•	•
Theiler's murine	•	•	•	•	•
Sendai virus	•	•	•	•	
Pneumonia virus of mice (PVM)	•	•	•	•	
Murine norovirus (MNV)	•	•	•		
Reovirus 3 (RF●3)	•	•	•		
Mouse rotavirus (EDIM)	•	•	•		
Ectromelia virus	•	•	•		
Lymphocytic choriomeningitis	•	•	•		
Polyomavirus	•	•	•		
Lactate dehydrogenase-elevating	•	•	•		
Mouse adenovirus (MAV1,	•	•			
Mouse cytomegalovirus (MCMV)	•	•			
K virus	•				
Mouse thymic virus (MTV)	•				
Hantaan virus	•				

IMPACT Rat Profiles

	IMPACT V	IMPACT VI
Mycoplasma spp.	•	•
Mycoplasma pulmonis	•	•
Pneumonia virus of mice (PVM)	•	•
Kilham's rat virus (KRV)	•	•
Toolan's H1 virus	•	•
Rat parvovirus (RPV)	•	•
Rat minute virus (RMV)	•	•
Lymphocytic choriomeningitis	•	•
Rat cytomegalovirus (RCMV)	•	•
Sendai virus	•	•
Rat coronavirus (RCV)	•	•
Sialodacryoadenitis virus (SDAV)	•	•
Seoul virus	•	
Mouse adenovirus (MAV1,	•	
Reovirus 3 (RF●3)	•	
Rat theilovirus (RTV)	•	

Appendix 20: Food/Bedding/Enrichment (2019-2020)

		Item #	Description	Use	PI
Bedding					
	Envigo RMS, Inc.	T.7070C	Certified Diamond Dry Cellulose	rats, mice as requested	
	(Teklad)	T.7090C	Certified Envigo Sani-chips	mice, rats	
	Shepherd Specialty Papers		ALPHA-dri certified cellulose bedding	Rats, mice as requested	
Diets					
	Envigo RMS, Inc.	T.2918.15	Irradiated Teklad Global 18% Rodent	rat	
		T.7913.15	Irradiated NIH-31 Modified 6% Mouse/Rat	mice	
		T.7913.CS	Irradiated NIH-31 Modified 6% Mouse/Rat	mice	
		T.7917.15	Irradiated NIH-31 Open Pellets	Dwarf mice	(b)(6)
		T.7917M.CS	Irradiated NIH-31 Open Meal	Dwarf mice	
		TD.06415	Irradiated Adjusted Calories (45 kcal/Fat)	rats	
	Research Diets, Inc.	212492i	Irradiated Adjusted Calories (60 kcal/Fat)	mice	
	Zeigler	420000-75-62	NTP 2000 Rod-Assay Wafer	mice	
Enrichment					
	Bio-Serv	S3472	Transgenic Dough, Bacon Flavor Diet, Sterile	breeding mice or rats	
		S3823P	Love Mash, Rodent Reproductive Diet, Sterile	breeding mice or rats	
		S5137	Sunflower Seeds, Black Oil, Sterile	breeding mice or rats, dermatitis	
		W0016	Manzanita wood gnawing sticks	Barbering, food grinding, etc.	
		S5769	Nutra-gel diet bacon tray- sterile	Prenatal, lactation, appetite stim.	
		S4798	Nutra-gel diet grain-base cherry tray- sterile	Prenatal, lactation, appetite stim.	
	Clear H_2O	70-01-1082	HydroGel®	Order & Use as Needed	
		72-04-5022	DietGel® Boost Irradiated	Order & Use as Needed	
		725-06-5022	DietGel® Recovery Irradiated	Order & Use as Needed	
		75-01-1001	MediDrop® Sucralose	Order & Use as Needed	
	SE Lab Group, Inc.	88-0001	Napa Nectar	Not currently in stock	
	The Andersons	BRN8SR	BedrNest Irradiated Certified	all mice	
		CNKR	Crink l'nest Kraft Irradiated	all mice	
	Shepherd Specialty Papers		Shepherd Shack®	Mice and rats	
			Shepherd Shack® Clear H2O Holder	Mice and rats	
	Ancare	NES3600	Nestlets	mice for nest building, comfort	

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Appendix 22: Animal Facility Equipment List

	Manufacturer	Model/Year	Description	# of cage spaces	# of units	Use
Animal Units						
	Allentown	2017-2019	NexGen Mouse IVC	140	15	Housing of mice
	Lab Products	2002	2002 model	140	1	Housing of mice
		RAIR/2013	RAIR mouse animal housing unit	140	3	Housing of mice
		RAIR/2015	RAIR Double-sided rat housing unit	48	3	Housing of rats
		RAIR/2015	RAIR Single-sided rat housing unit	24	3	Housing of rats
		RAIR/2016	RAIR Single-sided rat housing unit	24	1	Housing of rats
		RAIR/2018	RAIR Double-sided rat housing unit	48	4	Housing of rats
		RAIR/2019	RAIR Double-sided rat housing unit	48	1	Housing of rats
	Tecniplast	Blueline	Blueline Mouse Housing unit	144	8	Housing of mice
		Blueline newer	Blueline Mouse Housing unit	144	4	Housing of mice
	Thoren	2000	Thoren mouse 80 cage unit	80	1	Housing of mice
		1997-1999	Thoren mouse/rat 80 cage unit	80	1	Housing of mice/rats
		1997-1999	Thoren mouse/rat 70 cage unit	70	2	Housing of mice/rats
Workstations/Change stations						
	Lab Products	309098	Stay-clean work bench	n/a	4	Handling of animal cages
	Nuaire	NU-619-500	Animal Transfer (Cage Change) Station	n/a	1	Handling of animal cages
		NU-619-400	Animal Transfer (Cage Change) Station	n/a	1	Handling of animal cages
		NU-619-300	Animal Transfer (Cage Change) Station	n/a	2	Handling of animal cages
Cage Wash						
	AMSCO	Series 3043-S	Model 10E75W Pre-vacuum Sterilizer	n/a	1	Sterilize equipment & supplies
						(installed approx. 1995)
	MTP	Series 2200	Tunnel cage and utensil washer	n/a	1	Installed new in 1997
	Better Built	Model 6300	Rack washer	n/a	1	Reconditioned in 1998
	Nuaire	NU-607-400	Nuaire dump station	n/a	2	One purchased in 2016; one in 2017
Support Equipment						
	Kenmore	2016 and 2019	5 cubic ft. chest freezers	n/a	2	One for carcass storage; One for diet
		2018	19 ft. upright freezer	n/a	1	For carcass storage
			4 cubic ft. refrigerator		2	For diet storage
	Frigidaire	FFPE45L2QM	4 cubic ft. refrigerator	n/a	1	For diet storage
(b)(7)(E)	AIMS	LCS00	CO2 flowmeter	n/a	3	On individual CO2 cylinders
		SC100	CO2 flowmeter	na/	1	Hooked to house CO2

	Chapin		Hand held foamer/sprayer	n/a	1	For decontamination
	Dyna-fog		Cyclone Dyna-fog hand held fogger	n/a	1	For decontamination
	Flow Sciences		Ventilated necropsy work station	n/a	1	For in-house necropsy
	Ancare		Water bottle filler, dual header	n/a	1	(b)(7)(E)
	Avidity/Edstrom		3-stage microfiltration system	n/a	1	(b)(7)(E)
	Nilfisk	GM80/2017	2.5 gal HEP vacuum cleaner	n/a	1	Inhalation exposure rooms
		GM80/2007	2.5 gal HEP vacuum cleaner	n/a	1	Exp systems test lab, (b)(7)(E)
			Clean room HEPA vacuum cleaner	n/a	1	Animal breeding rooms
			Clean room HEPA vacuum cleaner	n/a	1	In Storage
	Shop vac		Shop vacuum, 5 gallon	n/a	2	One in AQ, One in storage
			Shop vacuum, 1 gallon	n/a	1	For small pick-ups
	Euthanex	M1-SBFF	Smartbox Auto CO2 System	n/a	1	Hooked to house CO2