Program Description
Animal Care and Use Program

National Institute
of
Environmental Health Sciences (NIEHS)

Research Triangle Park, NC

JULY 2017

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Program Description

Section 1. Introduction

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

The National Institute of Environmental Health Sciences (NIEHS), located in Research Triangle Park, NC, is the program unit seeking reevaluation of its intramural animal care and use program by AAALAC International.

NIEHS, one of the 27 institutes of the National Institutes of Health (NIH), is part of the NIH Intramural Research Program Office of Laboratory Animal Welfare (OLAW) Assurance but has a separate AAALAC accreditation from the Bethesda, Maryland based NIH.

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.

NIH is part of the Public Health Service within the U.S. Department of Health and Human Services, a cabinet-level department of the Executive Branch of the Federal Government. NIEHS is one of the NIH institutes, which are collectively headquartered in Bethesda, Maryland. The National Toxicology Program (NTP) is headquartered at the National Institute of Environmental Health Sciences (NIEHS). NIEHS is one of three core agencies that provide support for NTP activities. The mission of the NIEHS is to discover how the environment affects people to promote healthier lives

The NIEHS, like other NIH Institutes, has both an extramural and an intramural component. This Program Description relates to the NIEHS intramural research program. The NIEHS intramural research program plans and conducts basic, applied, and clinical research directed toward increasing fundamental knowledge of environmentally-related diseases and disorders. Broad multi-disciplinary research approaches are used including basic mechanistic studies at the cellular and molecular level, applied toxicology testing, and clinical and epidemiological studies. In pursuing their scientific endeavors, intramural scientists address a wide array of complex research issues including genetic susceptibility, receptor mediated pathobiology, understanding differentiation and development, signal transduction, environmental regulation of cell proliferation and cell death, environmental carcinogenesis and mutagenesis, environmental epidemiology, and immunology. These research endeavors, in turn, support biomedical and clinical program interests of the Institute.

The Comparative Medicine Branch (CMB) is within the Division of Intramural Research (DIR) and is responsible for providing clinical veterinary services, training for all personnel working with animals, animal husbandry, housing, enrichment, production, investigator surgical and technical support, administrative support for the Institute's Animal Care and Use Committee (ACUC) and compliance oversight. The branch also develops, refines, and advises Institute scientists of appropriate animal models for use in Institute research programs; procures experimental animals, tracks resource utilization by investigators including animal purchase and maintenance costs and special projects costs; maintains collaborative laboratories in microbiology and laboratory animal medicine; and plans and conducts research appropriate to these laboratory functions.

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. In the European Union, the standards applied might be the Guide, ETS 123, Directive 2010/63, and any country-specific regulations.

NIEHS uses the standards of the Guide, PHS Policy, and the Animal Welfare Act regulations for all animals. NIH Policy Manuals and Animal Research Advisory Committee (ARAC) Guidelines are also used.

D. Describe the organization and include an organizational chart or charts (as an Appendix/Appendices) detailing the lines of authority from the Institutional Official to the Attending Veterinarian, the Institutional ACUC/Oversight Body (IACUC/OB), and the personnel providing animal care. Please include the title, name (Note: For individuals whose information is publically available, provide the titles and names; for individuals whose information is not publically 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, 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 NIH Animal Care and Use (ACU) program is the responsibility of the Director of NIH, Dr. Frances Collins, who has appointed the Deputy Director for Intramural Research (DDIR), Dr. Michael Gottesman, as the responsible Institutional Official (IO).

The Deputy Director for Intramural Research/Institutional Official (DDIR/IO) is responsible for ensuring compliance with PHS and the NIH intramural policies by all intramural institute or center (IC) and others that use NIH facilities. The Policy Manual (PM) 3040-2: Animal Care and Use in the Intramural Program, delineates the responsibilities, interrelationships, and procedures followed within the intramural Animal Care and Use (ACU) program.

Director, Office of Animal Care and Use (OACU) has the authority delegated by the DDIR/IO for ensuring compliance of the Intramural ACU program with PHS and NIH policy, the Animal Welfare Regulations (AWRs), the provisions of the Guide, and other applicable policies, regulations, and standards.

The Director, OACU:

- maintains the NIH Assurance of compliance with Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy),
- reviews semiannual IC animal care and use program and facility evaluations for compliance with the Assurance, and forwards copies of the IC semiannual evaluations to the DDIR/IO.
- reviews animal facility construction and renovation plans to facilitate regulatory and compliance requirements,
- reviews and concurs with Interagency Agreements for establishing new animal program management oversight or facility support prior to their implementation,
- conducts unannounced site visits of animal care and use programs and facilities at the IO's request,
- acts on behalf of the IO to implement appropriate corrective actions within the NIH ACU program,
- compiles the annual NIH composite USDA, OLAW and AAALAC annual reports, and
- consults with IC ACUC Chairs and/or APDs on issues of potential non-compliance, and when issues are determined to represent serious or continuing non-compliance, reports these to the IO and OLAW. After preliminary reporting, tracks incidents, ensures their timely resolution and informs AAALAC.

Additional program authorities have been delegated to NIEHS per NIH Policy Manual 3040-2. Responsibility for the NIEHS animal care and use program is delegated to the Scientific Director (SD) in the Division of Intramural Research. The SD is advised by the NIEHS ACUC. Responsibility for implementation of the program and day-to-day management of the facilities is delegated to the Chief of CMB, a Supervisory Veterinary Medical Officer who serves as the Animal Program Director (APD) and Attending Veterinarian (AV). CMB is composed of the Office of the Chief (OC), Animal Care and Use Committee Office (ACUC), and sections for Veterinary Medicine (VMS), Quality Assurance (QAL), and Animal Resources (ARS). Daily animal care and husbandry, as well as rodent breeding support, are provided by a contract operation, FEFA. Organizational charts are provided in the appendices.

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.

Redacted by agreement

Dr. Kathy Laber, Chief, CMB, APD, AV

Redacted by agreement

Dr. Don Cook, Chair, NIEHS ACUC

Redacted by agreement

Redacted by agreement

F. Briefly describe the major types of research, testing, and teaching programs involving animals and note the approximate number of principal investigators and protocols involving the use of animals. As mentioned in the instructions, please complete one of the animal use forms included with this outline or provide the information requested in a similar format as an appendix.

NIEHS conducts a broad spectrum of research into genetics, cell signaling pathways, neurological development, and subsequent disorders, carcinogenesis, immunology, and infection, pharmacokinetics, reproductive development and diseases, epigenetic and stem cell biology, and toxicology, to better understand how environmental agents influence human health and disease.

There are 46 principal investigators (PIs) and 140 active animal study proposals (ASPs) at NIEHS.

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

NIEHS funding for animal research comes from congressional appropriation distributed to the Department of Health and Human Services (DHHS), to the NIH and then to the NIEHS. Funds are received by the NIEHS Budget Office and allocated to the various divisions.

H. List other units (divisions, institutes, areas, departments, colleges, etc.) of your organization that house and 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.

NA

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.

All IC animal activities involving the use of contract facilities are under IC ACUC oversight through the review mechanisms outlined in NIH Policy Manual (PM) 3040-3, "Intramural Acquisitions Involving Animal Research Activities".

Contracts are in place for animal procurement, customized breeding, and housing of mice, and specialized animal services (i.e. rederivation, embryo transfer, cryopreservation, speed congenics), with Taconic Farms, The Jackson Laboratory, and Charles River Laboratories. A contract is in place for generating transgenic and conditional knock-out mice with Taconic/Xenogen Biosciences. All these programs are PHS assured and AAALAC accredited.

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

NIEHS currently houses only mice and rats. Rodents housed at NIEHS are free of all known pathogenic agents including *Pasteurella pneumotropica*, *Helicobacter sp*. and Mouse Norovirus.

Redacted by agreement

Redacted by agreement Containment is at the room, cubicle, and cage level. Protective clothing (PPE) is changed between animal rooms.

The original accreditation of NIEHS occurred in 1972. Our animal care and use program has maintained continued full accreditation since its original accreditation. Following the last AAALAC site visit in 2014, NIEHS received continued full accreditation with no suggestions for improvement.

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 p. 13-14]
Describe how program needs are clearly and regularly communicated to the Institutional Official by the Attending Veterinarian, IACUC/OB, and others associated with the program.

The Attending Veterinarian (AV) reports directly to the SD and frequent face to face meetings occur to discuss animal program needs. In addition, the AV and the NIEHS ACUC chair meet semiannually with the SD to discuss animal program issues. Minutes from the monthly NIEHS ACUC meetings are sent to the SD.

The SD communicates frequently with the NIH DDIR and meets twice a month as part of a meeting of all NIH Scientific Directors.

The Director, OACU forwards all pertinent and critical reports and documents to the DDIR/IO for review and acknowledgement/approval: i.e. semiannual reports from all ICs; annual reports to OLAW, AAALAC, USDA; OLAW reportable events summaries and actions; Animal Research Advisory Committee recommended policy changes; recommended policy manual changes, etc. The Director, OACU meets regularly with the

DDIR/IO and keeps him apprised of general issues occurring within in the Intramural Research Program (IRP) ACU program. Additionally, senior leadership within the intramural ACU program can meet directly with the DDIR/IO to voice concerns or provide essential updates as felt appropriate.

Several NIH committees meet regularly to discuss NIH animal research issues. The NIH Animal Program Directors (APD) meeting is attended monthly by the NIEHS AV (who is also the NIEHS APD). This committee discusses issues which affect the overall animal care and use program. The NIH Animal Research Advisory Committee (ARAC) is advisory to the NIH DDIR and is comprised of all NIH ACUC chairs. This monthly meeting is attended by the NIEHS ACUC Chair or the AV to support the Institution's conformance to Guide recommendations. The Animal Facilities Program Advisory Board (AFPAB), composed of individuals representing the IC's and the Office of the Director and meets quarterly to prioritize proposed major renovation, construction, or maintenance and repair projects involving NIH animal facilities. Relevant information that results from these meetings is also relayed to both the IO and the SD for each institute.

b. The Attending Veterinarian [Guide, p. 14]

i. Describe the institutional arrangement for providing adequate veterinary care. For each veterinarian associated with the program (including private practitioners), provide the veterinarian's name(s), list responsibilities, and how the veterinarian is involved in monitoring the care and use of laboratory animals. If employed full-time by the institution, note the percentage of time devoted to supporting the animal care and use program of the institution. If employed part-time or as a consultant, note the frequency and duration of visits.

The Attending Veterinarian (also designated by NIH as the Animal Program Director) has direct line and program authority and responsibility for the health and well-being of all laboratory animals at NIEHS. As Chief of the Comparative Medicine Branch, the AV/APD manages a complete program of animal procurement, disease detection, control, surveillance, diagnosis, treatment, and preventive medicine. The AV/APD has appropriate authority and oversees all aspects of animal care and use through the CMB sections for Veterinary Medicine, Quality Assurance, and Animal Resources. The Attending Veterinarian is a member of the ACUC.

The AV and four additional full time veterinarians provide veterinary care as outlined below. Veterinarians have access to all animals and all medical records. Daily observation of all animals is an important component of the veterinary care program and veterinary care is available 24 hours a day, 7 days a week. The veterinarians, through CMB and its educational programs, provide guidance and monitoring of investigators on the use of anesthetics, analgesics, euthanasia agents, methods of animal handling and restraint, surgical techniques, and pre/post-surgical care.

Kathy Laber, DVM, MS, DACLAM, - 100% of time
Chief, CMB, Attending Veterinarian, Animal Program Director
Responsible for oversight and day-to-day implementation of the NIEHS intramural animal care and use program.
Manages the CMB
Reviews ASPs

ACUC member
Oversees and participates in training programs for veterinary residents, technicians, and animal care personnel
Provides clinical veterinary support to the animal program
Conducts collaborative research

Redacted by agreement

Oversees the VMS, QAL, and ARS
Reviews ASPs
ACUC member
Participates in training programs for veterinary residents, technicians, and animal care personnel
Provides clinical veterinary support to the animal program
Conducts collaborative research

Redacted by agreement

Responsible for the clinical health program at NIEHS
Coordinates pertinent diagnostic laboratory services (necropsy, histopathology, microbiology, clinical pathology, serology, parasitology)
Oversees surgery, rodent imaging, behavior phenotyping, and specialized technical assistance program for investigators
Provides guidance and monitors handling/restraint, anesthetics, analgesics, tranquilizers, and methods of euthanasia
Ensures appropriate training and proficiency
Monitors animal well-being before, during, and after experimentation
Pre-reviews ASPs
Provides training for veterinary residents, technicians, and animal care personnel.
Conducts collaborative research

Redacted by agreement

Responsible for the preventative health program through disease surveillance, prevention, detection, treatment, and resolution
Coordinates sentinel animal program and vendor surveillance program
Oversees testing and approves clearance of biologicals intended for use in animals
Furnishes diagnostic microbiology, serology, and parasitology services
Oversees testing of animal feed, bedding, and water
Provides clinical veterinary support to the animal program
Provides training for veterinary residents, technicians, and animal care personnel
Conducts collaborative research

Redacted by agreement

Supports preventive health program
Manages the sentinel animal program and assists with vendor surveillance program oversight
Coordinates all outgoing animal shipments
Provides clinical veterinary support to the animal program

Participates in training program for veterinary residents, technicians, and animal care personnel
Conducts collaborative research

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. An organizational chart depicting the reporting relationship between these individuals and the Attending Veterinarian should be included as an appendix.

The Animal Resources Program Manager, Animal Facility Manager, veterinary technicians, contract supervisory staff, and animal care technicians observe animals daily and consult with CMB veterinarians as necessary regarding animal health issues. Organizational Charts are provided in appendices three and four.

c. Collaborations [Guide, p. 15]
Describe processes for assigning animal care and use responsibility, animal ownership and IACUC/OB oversight responsibilities at off-site locations (i.e., collaborations).

Collaborations that occur at off-site locations are conducted under formal written agreement (contract, interagency agreement, or Animal Material Transfer Agreement (Animal MTA)).

All contracts require that the contractor be PHS assured and AAALAC accredited. Contract statements of work address responsibility of animal care and use, animal ownership, and IACUC review and are reviewed by the NIEHS ACUC. Animal care and use protocols at contract sites are reviewed by the contractor's ACUC and are deliverables to the NIEHS Contract Officer Representative (COR).

Interagency agreements and Animal MTAs specify transfer of animal ownership with the written agreement that the receiving institution will be PHS assured and follow national standards for humane care and use of animals, as described in the Guide and will comply with the Animal Welfare Act and its implementing regulations, as applicable.

2. Personnel Management

a. Training and Education
Describe how the IACUC/OB provides oversight and evaluates the effectiveness of training programs. Describe how training is documented.

All personnel working with animals are required to take the NIEHS Investigator training course "The Humane Care and Use of Animals in Research." This course is also taken by all ACUC members and is reviewed and updated on a regular basis. Documentation of course completion is maintained by the ACUC office and the training is documented on each person's training and experience (T&E) form, required during protocol review.

All personnel working with animals and ACUC members are also required to take an on-line triennial refresher course. This course is updated on a regular basis and reviewed by ACUC members. Documentation of course completion is maintained by the ACUC office.

The ACUC reviews the CMB Animal Husbandry Support Contract Statement of Work, which includes the requirement for a training program for animal care staff. Members of the ACUC and the CMB also actively participate in the contractor-training program.

Effectiveness of training programs is a topic for consideration during each ACUC semiannual program review. The NIH Office of Animal Care and Use also review the NIEHS training programs on a regular basis.

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

Provide name and credentials of veterinary and other professional staff, including the veterinary personnel listed above, and describe their qualifications, training, and continuing education. Please do not provide curriculum vitae of personnel.

Kathy Laber, D.V.M., M.S., DACLAM
Chief, CMB, Attending Veterinarian, Animal Program Director
33 years of experience in laboratory animal medicine
Licensed veterinarian in NC, SC, MI

Redacted by agreement

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Redacted by agreement

These personnel maintain appropriate current certification and licensure through membership and participation in professional organizations, participation in continuing education courses, and attendance at local and national meetings supported by CMB. In addition, the NC State College of Veterinary Medicine is near NIEHS and offers numerous continuing education opportunities for veterinarians.

ii. Animal Care Personnel [Guide, p. 16]
Indicate the number of animal care personnel: Redacted by agreement

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

Animal care is provided through a CMB Animal Husbandry Support Contract currently awarded to FEFA.

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Contract Animal Care Staff:

There are currently Redacted by agreement additional contract animal care employees with FEFA.

Of the total contract employees, Redacted by agreement have a college degree; Redacted by agreement with an AS, Redacted by agreement with a BA, Redacted by agreement with a BS, and Redacted by agreement with an MS. Redacted by agreement are AALAS certified LATG; Redacted by agreement are AALAS certified LAT; and Redacted by agreement are AALAS certified ALAT. Animal care staff has from 1-30 years of experience.

AALAS certification is required for all animal care staff except for the support operations staff (housekeeping, feed and bedding technician, and cage wash). Employees are supported in preparing and taking the AALAS examinations and are given one year from the time of employment to obtain the certification. Other incentives for obtaining certification include reimbursement of the exam costs, as well as a monetary bonus that increases with each level of certification obtained.

New contract employees are required to receive orientation and training in job related techniques and principles in basic animal care by the project management team beginning at the time of hire. Technicians are trained using an in-house prepared manual, which includes various SOPs, power point slides, information on normal and abnormal rodent behavior, common clinical signs and abnormalities broken down by body system. In addition, employees sign off on the SOP's that are pertinent to their position.

In addition, organized training is offered on a weekly basis and includes such topics as Health and Safety when working with research animals, zoonotic diseases, animal related allergies, basic animal behavior, Standard Operating Procedures (SOPs), and techniques and principles common to laboratory animal science. Training seminars are also given by CMB professional and technical staff, NIEHS scientists, NIEHS HSB staff and by invited outside speakers. All contract employees are required to attend the Humane Care and Use of Animals in Research course and an annual refresher training presented by CMB staff.

Program training is documented and maintained by the contract Training Specialist. Institute training records (IT, Security Awareness etc.) are maintained with the contract management staff. The ACUC office maintains records of attendance at the CMB Seminar on The Humane Care and Use of Animals in Research.

Contract personnel are strongly encouraged and supported to attend continuing education activities and to participate in local and national laboratory animal science

and other relevant professional organizations. Employees are encouraged and supported to attend AALAS classes at the appropriate technical level and are counseled and encouraged to attend classes at local community colleges or universities to update skills or further their education. Tuition assistance is available. An annual travel budget of approximately $20K is available to allow employees to attend off site meetings, such as National AALAS, LAWTE, Jackson Laboratory's Colony Management Course, ILAM, The Charles River Short Course, PRIMR, IACUC 101 and IACUC Advanced, NCAB AALAS, District IV AALAS, as well as other relevant meetings and training opportunities.

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 members of the research staff working with animals, including PIs, staff scientists, research technicians, trainees, students, and visiting scientists are required to complete the NIEHS Investigator training course "The Humane Care and Use of Animals in Research". Documents referenced in the training are provided as links to the CMB website. A triennial refresher course is also required. Hands-on training and specialized training relative to studies are available from the VMS. Verification of all relevant training and experience is reviewed by the ACUC during protocol review. VMS monitors competency in new procedures documentation of proficiency is submitted to the ACUC office.

a) Briefly describe the content of any required training.

The NIEHS investigator training course " The Humane Care and Use of Animals in Research" reviews pertinent legislation and regulations applicable to the use of animals in research, the ethics of animal use and the principles of the 3 R's, the function of the NIEHS ACUC and the animal care and use program, basic animal handling, ways to minimize pain and distress in laboratory animals, anesthesia, analgesia, aseptic surgical techniques, appropriate euthanasia, occupational health and safety in the research facility, allergens, zoonotic agents, use of personal protective equipment, and mechanisms for reporting concerns. Upon completion of the training course, investigators are required to complete a facility orientation with a member of the CMB staff.

An on-line refresher training course, highlighting the topics in the "Humane Care and Use of Animals in Research" is required on a triennial basis for all NIEHS employees working with animals.

In addition, the ACUC may require or the PI may request hands-on training in animal handling and technical procedures. This may be presented in a small group setting or on a one-on-one basis and is instructed by CMB veterinarians or trained VMS technicians.

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

All animal users must have adequate training before beginning animal work. "The Humane Care and Use of Animals in Research" training is available on-line and must be completed prior to any animal user being added to an animal study proposal.

c) Describe continuing education opportunities offered.

Continuing education opportunities (IACUC 101, IACUC Advanced, NCABR meetings, OLAW webinars), updates on legislation, availability of animal models, use of alternatives, advances in relevant technology, or changes in the NIEHS animal care and use program are communicated to animal research personnel by CMB or the ACUC as information becomes available. New and revised SOPs are reviewed by the ACUC and are available on the CMB web pages. Specialized up-to-date technical training by the CMB VMS is available to all personnel.

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

Researchers and technical staff must have appropriate training to ensure that good surgical technique is practiced. Basic surgical principles such as asepsis, animal preparation, sterilization of instruments, use of anesthetics and analgesics, and appropriate animal monitoring are discussed in the required "Humane Care and Use of Animal in Research" training course.

The VMS has developed SOP's for all surgical procedures commonly performed at NIEHS. VMS develops, refines, and performs most surgical procedures for investigators. Investigators may work with the VMS as new surgical procedures are developed for their use and gain training and experience during this part of the process. All survival surgical procedures are conducted in the animal facility under oversight of the VMS.

All surgical procedures are either described in the ASP or the VMS SOP for a surgical procedure is referenced. Training and experience of the person(s) performing the surgery must be documented in the T&E which is maintained by the ACUC office. Surgeons must be listed on the ASP. The ACUC reviews the ASP and gets confirmation that the surgeon has been adequately trained via the T&E form. If the VMS does not have direct knowledge of the surgeon's experience with a procedure, the ACUC will require that the surgeon be assessed by the VMS the first time the procedure is performed. VMS will work with the surgeon, provide training if necessary, and monitor performance until qualifications are met. Documentation of the completed assessment is filed by the ACUC office as a memo to the record and the surgery is added to the surgeon's T&E.

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

Anesthetics are administered by CMB veterinarians, trained animal technicians, or by qualified investigators and research technicians.

Appropriate use of anesthetics and animal monitoring are covered in the required "Humane Care and Use of Animals in Research" training course. Guidance has been developed for the selection and proper use of analgesic and anesthetic drugs by VMS and is reviewed and updated on a regular basis, and is available to all investigators on the CMB web pages.

As stated above, VMS will work with the surgeon in anesthetic applications, provide training if necessary, and monitor performance until qualifications are met. Selection of appropriate anesthetics and analgesics and training and qualifications of the investigator are reviewed by the ACUC during ASP review.

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

Proper euthanasia techniques are covered in the required training course "The Humane Care and Use of Animals in Research." Euthanasia methods used at NIEHS are consistent with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition and are reviewed and approved by the ACUC in each ASP. In addition, standardized methods of euthanasia have been developed and approved by the AV and ACUC and include Carbon Dioxide Euthanasia of Rodents, NIEHS Euthanasia Methods for Rodent Fetuses and Neonates, and Decapitation Procedures for Mice and Rats.

It is essential that skilled personnel perform euthanasia in a professional and compassionate manner and that death be assured. CMB staff performing euthanasia are trained and assessed by VMS. CMB contract staff performing euthanasia are trained and assessed by contract supervisory staff. Euthanasia SOPs are reviewed as part of ongoing staff training. Instructions for proper CO_2 euthanasia are posted in each procedure/necropsy room in the animal facility.

Qualified ASP participants or VMS staff train research staff in performing euthanasia. Instructions for proper CO_2 euthanasia are posted in each laboratory approved for use of this procedure.

VMS assesses anyone performing euthanasia using physical methods without anesthesia to ensure proper procedures and technique. Documentation of proficiency is filed with the ACUC office on the T&E and/or by memo to the record (MTR). An SOP for decapitation procedures for mice and rats is distributed to those utilizing this method of euthanasia. A discussion of procedures and an assessment of the maintenance of guillotines and scissors are completed during the semiannual laboratory visits.

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

Describe the institutional entities that are involved in the planning, oversight, and operation of the institutional occupational health and safety program.

The NIEHS Health and Safety Branch (HSB), which is administratively located within the Office of Management (OM), has responsibility for the planning, oversight, and operation of the NIEHS occupational health and safety program (OHSP). Key personnel in the HSB and programmatic responsibilities are as follows:

Redacted by agreement

The oversight of the use of hazardous agents at NIEHS is accomplished using safety protocols. PIs are required to prepare a safety protocol when hazardous agents (biological, chemical, or physical) are to be used in research projects. The Laboratory/Branch Chief reviews the protocol prior to submitting it to the HSB for review and approval by the appropriate safety protocol review committee:

*Biological agents, Human Materials, and Recombinant DNA – NIEHS Institutional Biosafety Committee (IBC)
*Chemical agents – Hazardous Agent Protocol Review Committee
*Radioactive materials – Radiation Safety Committee
*Lasers – Radiation Safety Officer & Health Physicist

Safety protocols for the use of hazardous agents (biological, chemical, or physical) must be approved prior to ASP/amendment approval by the ACUC. A HSB representative serves as Ex-Officio ACUC member to ensure coordination between the ACUC and the HSB.

ARS is involved in the oversight and coordination with HSB on the use of hazardous agents in the animal facilities. Prior to the initiation of any new study, an experimental summary sheet is submitted by the lab to ARS which details dosing, special husbandry, and safety requirements. The experimental summary sheet is reviewed to ensure compliance with HSB recommendations found on the safety protocol animal addendum.

The Animal Husbandry Support Contractor, FEFA, supplies its own occupational health and safety program through Duke Occupational Health that meets or exceeds the

requirements of the NIH Occupational Safety and Health Program and all applicable sections of the Occupational Safety and Health Act of 1970, as amended (U.S. Code of Federal Regulations, Title 29). The contractor's program is monitored by the NIEHS Contract Officer's Representative (COR).

i. **Hazard Identification and Risk Assessment** [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]

1) Describe the process used to identify, evaluate and control experimental and other potential hazards (such as ionizing and non-ionizing radiation, chemical cleaning agents, animal bites, allergens, zoonoses, and venomous species) inherent or intrinsic to the use of animals by the institution. Describe how risks of these hazards are assessed and how procedures are developed to manage the risks.

The NIEHS has implemented a safety protocol review process that is a critical component of our risk assessment and hazard identification efforts. PIs are required to prepare a safety protocol when hazardous chemicals, biological agents including human materials or Recombinant DNA, radioisotopes, or Class 3B or Class 4 lasers are to be used in research projects. The process of completing the safety protocol requires researchers to self-evaluate their practices and personally commit to safe and responsible procurement, use, and disposal of the materials. Safety protocols include an animal addendum that identifies specific hazard controls relating to the care and use of study animals. The safety protocol and animal addendum is reviewed and approved prior to approval of the ASP and initiation of animal work.

In addition to an annual collaborative workplace safety walkthrough (encompassing chemical safety, radiation safety, biological safety, environmental protection and industrial hygiene) inspection of all facilities, the HSB has developed programs for monitoring occupational exposures. Workplace air samples for evaluating a variety of occupational exposures such as organic vapors, waste anesthetic gases, dust and other potential workplace contaminants are routinely collected. Other special monitoring studies are conducted as appropriate (e.g., lighting levels, noise, personnel heat stress, etc.).

The number of animal studies at NIEHS that use radioisotopes is small. These studies require an approved safety protocol and are subjected to the same rigorous radiological surveillance programs as required in other laboratories using radioisotopes. Animal studies are confined to Illinois cubicles and containment rooms, which are monitored routinely by the investigator during the study and at its completion. Monitoring is also conducted by the HSB at a minimum of once per month or more frequently if a problem arises. Redacted by agreement

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Class 3b or Class 4 open beam lasers require active laser protocols. Laser protocols are reviewed to assure proper protection from potential hazardous non-ionizing radiation. Where possible engineering practices are used to contain and direct the radiation. Personnel protection is achieved with laser goggles or glasses.

Animal biosafety level (ABSL) designation criteria are discussed in laboratory health and safety training conducted by the HSB.

Zoonotic diseases are discussed in the CMB course "The Humane Care and Use of Animals in Research".

Minor animal bites or scratches can be evaluated and treated in the NIEHS Health Unit.

The NIEHS Health and Safety Guide to Laboratory Ergonomics was developed by the HSB and distributed to all laboratory personnel and made available on our internal website at http://junction.niehs.nih.gov/divisions/management/safety/manuals/ergonomics_guide/index.htm. This guide helps research staff reduce and eliminate repetitive motion injuries and awkward postures in the laboratory environment by incorporating simple ergonomic principles. HSB staff performs ergonomic assessments upon request and will make changes and modifications as required to eliminate repetitive motion injuries in all NIEHS facilities.

The use of cleaning agents is reviewed from both an occupational and environmental health and safety perspectives by the HSB before any agent can be used in the animal facility.

The NIEHS Health Unit maintains a listing of immunizations available to personnel and are discussed in the Laboratory Health and Safety Course.

NIEHS personnel involved in the use of animals are required to have an individual risk assessment. and enrollment in the occupational health surveillance program. Employees complete a health history that asks questions about pre-existing animal allergies during enrollment in the Occupational Health Program.

All personnel handling animals must attend the Laboratory Health and Safety course and complete the Humane Care and Use of Animals in Research course where they are advised of potential hazards in the research laboratory including animal allergens and educated about respiratory equipment and the need to contact the HSB if problems arise.

2) Describe procedures for reporting and evaluating exposure to hazards, work place injuries, etc.

As part of their initial health and safety training, employees are instructed to report observed hazards and exposure incidents to their supervisor and the HSB. Employees are to report all work-related injuries and illnesses, regardless of severity via the Employees' Compensation Operations and Management Portal (https://www.ecomp.dol.gov) This portal is a web-based application for government-wide use that records workplace injuries and illnesses. This information is received by the HSB and is used in evaluating and controlling hazards, fulfilling mandatory recordkeeping requirements, and providing workers' compensation benefits.

The NIEHS Health Unit [Redacted by agreement] is available for provision of first aid for minor injuries. The NIEHS maintains a 24-hour emergency phone number [Redacted by agreement] that employees use to notify Security when immediate assistance is needed for a medical emergency, hazardous material spill, or other emergencies. Upon notification, Security officers respond to the scene and issue emergency radio calls to activate the appropriate response team (e.g., medical and/or hazardous material). These teams receive on-going training in emergency response skills and techniques.

The HSB investigates all injuries at NIEHS. The accident evaluation focuses on identifying the human techniques and activities and uses of equipment or instrumentation involved in the accident; the circumstances and underlying factors leading to the accident; and the methods or actions to prevent similar events.

Post-exposure medical surveillance is initiated depending on the nature and possible severity of the incident. Medical follow-up protocols have been established for certain types of incidents, such as potential bloodborne pathogen exposures.

Each calendar year, the HSB prepares a summary report that describes the incidence of work-related injuries and illnesses that have occurred at the Institute. OSHA recordable injuries and non-recordable "near miss" incidents are included in the analysis to identify trends and accident prevention opportunities.

ii. Facilities, Equipment and Monitoring [Guide, pp. 19-20]

1) Describe how hazardous agents are contained within the study environment and in the animal housing area.

Containment is required for all hazardous substance animal studies and is identified during review of the safety protocol. Containment may be one or more of the following items:

- Cage Filter Top
- Ventilated Cage Rack
- Illinois Cubicle
- Biological Safety Cabinet
- Chemical Fume Hood

- Cage Dump Station
- Room air flow balance that creates room pressure negative to the adjacent corridors and rooms.

2) Describe facilities that use hazardous agents. Note square feet/meters, number of animal rooms, and support spaces. In addition, describe design features, construction features, and special equipment, especially as they relate to hazard containment. Note if, and how, exhaust air is treated. If special facilities are not available and animals exposed to hazardous agents are housed within conventional animal rooms, so note.

Animals are dosed in a chemical fume hood or biological safety cabinet unless otherwise specified. Specific areas are provided and identified in each module for hazardous substance use and safety protocols and appropriate signs are posted. Animals are held in containment rooms (HEPA filter exhaust) equipped with ventilated cage racks or Illinois cubicle room, if deemed appropriate by HSB, for the required time or duration of the study.

Illinois cubicle and containment room walls are constructed of cinderblock, sealed, and covered with epoxy paint. Floors are concrete covered with sealed epoxy. All containment and Illinois rooms are equipped with chemical fume hoods and/or biological safety cabinets. All rooms have a minimum ventilation rate of 10 air changes/hour. Room pressure is negative to both clean and return corridors when used for containment purposes. Exhaust air leaving the containment rooms is filtered through a pre-filter and HEPA filter:

Illinois Cubicle Rooms (net square feet):

Redacted by agreement

Redacted by agreement

Containment Rooms (net square feet):

Redacted by agreement

Procedure/Necropsy Rooms (net square feet):

Redacted by agreement

[a] Rooms are dedicated with fume hoods, have stainless steel sinks, and counter tops for work with radioactive materials.
[b] Rooms are equipped with fittings to duct the exhaust from ventilated cage racks directly into the buildings exhaust system.
[c] Air is removed from the cubicles, fume hood, and room and flows to one of two sides of two redundant bag in-bag out filter housings. The filter housings contain both a HEPA filter and carbon filter to remove contaminants. Each housing can be isolated for maintenance without a loss of filtered exhaust from the room. The air flow continues through stainless steel or epoxy coated duct work, through additional HEPA filters before entering the exhaust fan and exiting the roof through exhaust stacks discharging at approximately 13 feet above the roof line.

3) Describe the oversight process and husbandry practices in place to ensure personnel safety, including any personal protective equipment provided when work assignment involves hazardous agents.

Prior to initiating an animal study involving hazardous agents, a safety protocol must be approved by the HSB, and an ASP/amendment must be approved by the ACUC. The ASP/amendment is not approved until a safety protocol with a safety protocol animal addendum is approved by the HSB. The safety protocol specifies handling of animals, cages and waste materials, special husbandry requirements, and PPE required to ensure personnel safety. Investigators are responsible for assuring that safety precautions contained in approved safety protocols are followed.

Prior to the initiation of any hazardous study involving animals an experimental summary sheet (ESS) is submitted to the Animal Facility Manager. The facility manager reviews the ESS for congruency with the ASP, safety protocol, and safety protocol animal addendum and coordinates a pre-study meeting attended by the appropriate scientific staff, husbandry staff and veterinary medicine staff to ensure that all safety, husbandry, and animal health issues are clearly defined. The ESSs are maintained at the animal room level. A warning placard using pictograms and indicating general occupational health requirements, personal protective equipment requirements, animal biosafety level, the PI and emergency contact information, and required procedures for entering and exiting the animal area is placed on the door when the study is in progress.

Investigators, research technicians, and animal care employees assigned to studies using potentially hazardous agents are required to wear PPE that protects against the risk identified. These can include respirators, masks, gloves, goggles,

or face shields as stipulated by the safety protocol. Employees are fit tested prior to the use of any respirators when required.

All work surfaces (bench tops, hood surface, etc.) where toxic substances, chemical carcinogens, or biohazardous materials are used are covered with stainless steel, dry absorbent plastic backed paper or other impervious material. The protective surfaces are decontaminated or disposed of after the procedure involving a toxic substance has been completed.

No animal rooms are used for preparation of dose solutions or feed/water stocks containing test compounds.

Studies involving chemicals that are likely to bond to or be incorporated into the animal cage plastics, or cause difficulty in cleaning as judged by the HSB, are conducted in disposable animal cages.

Animal studies utilizing toxic substances are conducted only in areas designated as suitable and approved by the HSB. Studies using radioisotopes are only conducted in animal rooms designated for this purpose.

Dead animals are disposed by double bagging, boxing, and labelling for incineration. All hazardous wastes including carcasses are separately packaged and labeled for pick up by the HSB. Chemical waste is yellow bagged, biohazardous waste is orange/red bagged, as appropriate for the hazard. For biohazardous material, the safety protocol addresses any special procedures (i.e., disinfection, autoclaving etc.) that are required to prepare the material for pickup and disposal.

4) Describe any facilities that may also be used for 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; the NIEHS Clinical Research Unit (CRU) is located in a separate building on the NIEHS campus. Animals never enter this building.

5) Describe any other circumstances in which animals or caging equipment are transported in common use corridors (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.

Incoming animals are transported into the NIEHS animal facilities via a common use elevator in filtered animal crates.

Animals may be transported in caging from the NIEHS animal facility to laboratories in the same building by CMB personnel or by approved laboratory personnel. When animals must be transported through common hallways or elevators, care is taken to assure that all cages are covered with a filter top at all times.

Cages with animals dosed with biological agents are provided secondary containment prior to transport.

6) If motorized vehicles are used for animal transport, describe how the driver is protected from exposure to hazards such as allergens or zoonoses.

All local off-site transportation of animals is performed by ARS personnel using appropriate containment (filtered crates or filter-topped cages) and a dedicated climate-controlled vehicle. A filter minimizes exposure of the driver to allergens or zoonotic agents. After returning from each delivery of NIEHS animals, the CMB van is sanitized using a QAL approved disinfectant. A more extensive sanitation regime is performed weekly (see SOP- Sanitization of the CMB Animal Transport Van).

iii. Personnel Training [Guide, p. 20]

1) Describe educational program(s) to inform personnel about zoonoses, personal hygiene, allergies, and other considerations regarding occupational health and safety.

HSB offers a variety of Health and Safety Training courses for NIEHS personnel. These include:

Introduction to Health and Safety at NIEHS: Required for all new NIEHS personnel as of June 2017. This course includes general safety training such as shelter in place, evacuation, ergonomics, HAZCOM and safety data sheets, general waste disposal guidelines, electrical safety, slips/trips and fall avoidance as well as incident reporting requirements and procedures. It also outlines the content for the more specific training courses.

Laboratory Safety at the NIEHS: This course, or an approved substitute, is required for all laboratory workers at NIEHS. Course materials include; more specific guidance to laboratory workers on general safety as well as detailed overviews of the biological, radiation and laser safety programs, ventilation, proper use of biological safety cabinets, chemical storage and use, cryogenics and compressed gases.

Biological Safety at the NIEHS: Required for all laboratory personnel utilizing human blood/tissues/fluids or recombinantR RNA in their research. Course materials include; Bloodborne pathogen training and provides an overview of all aspects of the NIEHS requirements for research involving human blood, fluids, and tissues. A bloodborne pathogen refresher course is required for laboratory and animal care personnel annually.

Radiation Safety at the NIEHS: Required for all personnel utilizing radioactive materials in their research. Outlines the detailed and specific requirements to safety use radioactive mateials at NIEHS such as contamination surveillance, controlling materials, risk to human health and "As Low As Reasonably Achieveable" (ALARA) concepts.

Laser Safety at the NIEHS: Required for all personnel utilizing lasers in their research. Outlines the detailed and specific requirements to safety use lasers at NIEHS such as; biological effects from exposure to laser light, laser regulations & standards, laser classifications and emergency procedures.

In addition, routinely scheduled courses in CPR/AED, First Aid, and Fire Extinguisher Use are made available to all interested NIEHS employees and contractors. The HSB also provides other special training such as chemical hazards for animal care personnel, safe lifting for warehouse and animal care personnel, and use and care of compressed gas cylinders for various Institute groups.

The HSB also provides a brochure on animal allergens "Allergies in the Research Laboratory" to all employees. A reproductive and developmental protection program is available for all employees. Employees may request the HSB to evaluate working areas and duties for potential reproductive hazards. Other special considerations are addressed during the protocol review process and referred to the occupational health physician for medical consultation or evaluation.

CMB offers:

The Humane Care and Use of Animals in Research: Required for all personnel using animals in research. Includes information about zoonotic diseases, allergies, personal hygiene, PPE, handling of animal-related waste materials, bites and scratches or other animal related injuries, and enrollment in the Occupational Health Program.

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

All government laboratory personnel and those using animals are required to attend the course "Introduction to Health and Safety at NIEHS" which provides information on hazard communication procedures, bloodborne pathogens, zoonoses, and laboratory safety policies. The "Laboratory Health and Safety" course, required of all laboratory personnel working with hazardous material, includes instruction in basic toxicology, personal protection, chemical hazards, containment equipment and procedures and housekeeping. Employees must also complete an on-line "Laboratory Refresher Course" every five years. If the animal study involves the use of human blood, human cell lines, human tissues, or human organs, then annual bloodborne pathogen update training is required of those research personnel. In addition, all employees working with radioactive materials are required to attend the NIEHS "Introduction to Radiation Safety" course, which covers physical characteristics of radiation, dosimetric quantities, biological effects and risks of radiation exposure and radiation safety procedures. All users of radioactive material are required to receive radiation safety refresher training every two years. In addition, the contractor conducts specifically designed in-house training sessions for animal care personnel that emphasize

proper use and types of containment, personal protection, hazardous waste disposal, Safety Data Sheet (SDS) training, zoonotic diseases, and laboratory animal allergens.

NIEHS operates a variety of data systems that provide employees with information on toxic substances. Information on Hazardous Agent Protocols is computerized and updated as necessary. The HSB maintains a computerized SDS database request and tracking system at https://apps.niehs.nih.gov/hsb/msds/listing.cfm that is accessible to employees and contractors. Links to other SDS websites are also provided.

iv. Personal Hygiene [Guide, p. 20; Ag Guide pp. 4-5]

1) List routine personal protective equipment and work clothing provided for animal care personnel, technical staff, farm employees, etc. Describe arrangements for laundering work clothing.

Animal care personnel are required to wear a cotton/polyester two-piece uniform of scrub pants and a long sleeved, snap-front shirt, which are commercially laundered, when working in animal rooms. Animal care personnel enter locker rooms; remove street clothes, don clean uniform, head cover, safety shoes, shoe covers, mask, and gloves to enter the clean corridor. Animal care staff are required to wear eye protection when not working in a change station.

Support area personnel working in the cage wash are required to wear clean uniforms, head covers, safety shoes, gloves, safety glasses, surgical mask (clean cage wash)/fit-tested N-95 particulate respirators (soiled cage wash) and protective hearing devices as stipulated by the HSB. Personnel working in clean cage wash wear shoe covers.

Investigators, research technicians, and animal care employees entering non-hazardous work areas wear disposable laboratory coats, surgical masks, head covers, shoe covers, and gloves.

Investigators, research technicians, and animal care employees assigned to studies using potentially hazardous agents are required to wear disposable coveralls or disposable laboratory coat, surgical masks (or respiratory protection as identified in the safety protocol and safety protocol animal addendum), shoe covers, head covers, gloves, and eye protection as determined by HSB dependent upon the hazard and stipulated by the safety protocol. Employees are fit tested prior to the use of any respirators when required.

Stations stocked with the appropriate PPE for entering the animal rooms (disposable laboratory coats/coveralls/gowns, shoe covers, head covers, surgical masks/N-95 particulate respirators, gloves, and disposable safety eye glasses) are in return corridors at the entrance to the animal rooms. PPE is required to be worn by ALL personnel entering an animal room and is removed and disposed of after leaving the animal room. Written instructions are issued by the HSB when special requirements are necessary such as special handling of disposable clothing. PPE is completely changed between animal rooms.

No PPE worn in an animal room is worn outside of the animal facility.

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

All personnel must wash their hands immediately after the completion of any procedure in which a hazardous material (eg, toxic or carcinogenic chemical) has been used. Sinks are available in all animal and procedure rooms. Immediately after any known exposure, employees must wash and shower and notify their supervisor and the HSB.

Hand sanitizers are also located throughout the animal facility and at each elevator vestibule.

Separate shower and change facilities are provided for men and women in the animal facility. All areas contain bathrooms, locker areas, individual shower stalls and antechambers that exit to the clean corridor. Hand and body soap is provided in these areas. Clean side antechambers are stocked with disposable lab coats/coveralls, head covers, shoe covers, surgical mask, and gloves.

Redacted by agreement

Uniforms may not be worn outside the animal facility/animal support areas.

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

NIEHS is a tobacco free campus and smoking is prohibited throughout the facilities. Eating, drinking and the storage of food are prohibited in all areas of the animal facility except in offices and the specialized break areas provided with tables, chairs, sinks, microwave ovens, refrigerator, vending machines, and drinking water.

Employee Lounge/Break Areas – Redacted by agreement

v. Animal Experimentation Involving Hazards [Guide, pp. 20-22]

1) Describe briefly institutional policies governing experimentation with hazardous biological, chemical, and physical agents, including the oversight process for the use of hazardous agents. Note: Written policies and standard operating procedures (SOPs) governing experimentation with hazardous biological, chemical, and physical agents should be available during the AAALAC site visit. If such policies and procedures are not available, please explain.

Institute health and safety policies and procedures are contained in the NIEHS Health and Safety Manual and the NIEHS Waste Manual. The current versions of all manuals can be found on the HSB website, which is accessible to all Institute employees.

The primary emphasis of the NIEHS health and safety program is exposure prevention through proper experimental design and use of laboratory containment equipment. Personal protective equipment is used to supplement other preventive measures.

The oversight of the use of hazardous agents at NIEHS is accomplished through safety protocols. The protocol identifies the type and degree of hazard, in addition to the necessary containment equipment and PPE. Decontamination, waste disposal and emergency procedures as well as special storage conditions are also described in detail. Finally, the extent and frequency of medical surveillance is based on a review of the submitted protocol. NIEHS safety protocols are required for all research where hazardous, biological, chemical, and physical agents are used:

The primary responsibility for completion of the appropriate safety protocol lies with the PI. The Laboratory/Branch Chief reviews the protocol and must approve it prior to submitting it to the HSB and the appropriate Protocol Review Committee. The Safety Officer, Biological Safety Specialist, or the Radiation Safety Officer coordinates the review process, consults with researchers, arranges for medical surveillance, inspects the facilities and equipment, and determines the adequacy of the safety measures. The HSB maintains the protocol through an internal database and reviews the approvals annually. Once the protocol is approved by the Hazardous Agent Protocol Review Committee, IBC, or the Radiation Safety Committee; the Safety Officer, Biological Safety Specialist, or Radiation Safety Officer issues the official approval and the employees performing the work are supplied with a copy of the protocol.

All safety protocols must be resubmitted for review and approval at least every three years.

The committees and teams that review the safety protocols critically evaluate the adequacy of seven primary factors associated with the experiment:

- Experience and training of the personnel
- Proposed experimental techniques
- Containment facilities
- Personal protective measures
- Decontamination procedures
- Waste disposal procedures
- Emergency contingency plans

For studies involving animals, a copy of the approved safety protocol is sent to the ACUC office by the HSB. The ACUC assures that the appropriate safety protocol has been approved prior to ASP approval. This is also verified at the animal facility level during the pre-study meeting that occurs before initiation of a hazardous study.

Use of Recombinant DNA Molecules

All research proposals involving the use of recombinant DNA molecules are assessed for conformance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Animal experiments involving recombinant DNA cannot be initiated until the PI receives confirmation that the work has been approved and registered with the NIEHS IBC. The PI is responsible for compliance with the Guidelines in the conduct of rDNA research, and for ensuring that appropriate reviews and approvals are obtained prior to initiation of experiments. The NIEHS IBC, whose functions are defined under the Guidelines, reviews and approves research protocols involving the use of rDNA techniques or potentially infectious/toxic materials. Recombinant DNA registrations are maintained on an internal database and copies are sent to the PI and CMB. The PI in collaboration with HSB develops a safety protocol animal addendum. The ACUC assures that the Recombinant DNA registration and safety protocol have been approved prior to ASP approval.

It is the responsibility of the PI to provide the IBC with updated research information, as well as other changes to laboratory location and research personnel. All rDNA registration documents are updated annually.

2) Describe aspects of the health and safety program specifically for personnel potentially exposed to hazardous agents.

All NIEHS personnel participating in research projects involving animals are medically evaluated by the Occupational Health Unit and cleared to work with animals. All contract animal care staff must enroll and be cleared through the contractor's occupational health surveillance program. Short-term workers (e.g.,summer students) must ensure that their personal physician completes an individual health and job related risk assessment (medical clearance form) and determines that the individual can safely work with animals. The medical clearance form provided must be returned signed by the physician to the occupational health unit to allow access to the NIEHS animal facilities.

Based on medical history and risk assessment, appropriate PPE, additional surveillance measures, and biological indicators of exposure may be initiated.

As part of their initial health and safety training, employees are instructed to report observed hazards and exposure incidents (suspected or overt) to their supervisor and the HSB. Post-exposure medical surveillance is initiated depending on the nature and possible severity of the incident. The HSB will perform an evaluation of reported incidents to determine the estimated potential for exposure to any potential agent used in an animal study.

A pre-study meeting is required for studies involving hazardous agents that have been previously approved by the ACUC. This meeting is attended by investigative staff, VMS staff, the animal facility manager, and animal husbandry technicians and outlines the safety requirements as well as experimental design and endpoints, humane endpoints, and supportive husbandry procedures.

There may be other instances where the HSB will conduct air sampling studies to quantify any possible employee exposure to ensure containment equipment is operating efficiently.

3) Describe safety procedures for using volatile anesthetics and how waste anesthetic gases are scavenged.

All volatile anesthetics must be appropriately scavenged which may include the use of a fume hood (available in procedural areas, containment rooms, Illinois cubicles) or a scavenging device (i.e. F/air™ canister). The HSB monitors the effectiveness of scavenging procedures utilized. The most recent survey of portable anesthetic gas scavenging systems, completed by the HSB in June 2017, determined that personal samples were below the NIOSH recommended exposure limit. All vaporizers are serviced every three years. An SOP for the use of the portable isoflurane anesthesia system in rodents was last reviewed June 2017.

4) 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: This information may be provided as an Appendix.

a) Biological agents, noting hazard level (CDC Biohazard Level, Directive 93/88 EEC, CDC or USDA/DHHS Select Agent, etc.).

see appendix

b) Chemical agents, noting general category of hazard (toxicant, toxin, irritant, carcinogen, etc.).

see appendix

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

see appendix

5) Describe the program for housing and caring for animals exposed experimentally to the hazardous agents noted above, with emphasis on management and safety

practices for containment of each class of agent. Indicate how levels of personnel exposure are assessed.

Through the safety protocol review and approval process, potential hazards and health and safety risks associated with exposure to the agent in question and/or contaminated animals are identified. The safety protocol outlines the containment procedures and the appropriate PPE to use when dosing and handling contaminated animals and waste materials. HSB reviews the safety protocol to assess the level of potential personnel exposure, PPE requirements, containment equipment to be employed, taking into consideration the agent used, dose, route, volume, frequency, duration of administration, metabolic products, routes of excretion, species, and number of animals.

All health and safety protocols are reviewed on an annual basis to ensure that they are current with respect to personnel involved, the agents/doses used, and number of animals.

Radioisotope levels of exposure are monitored using personal dosimeter badges. Employees are instructed to report exposure incidents to their supervisor and the HSB.

vi. Personal Protection [Guide, pp. 21-22]

1) Describe training, 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).

At NIEHS, training courses teach employees to recognize potential hazards to prevent or reduce injury. This training includes, but is not limited to:

Occupational Safety and Health Administration (OSHA) Laboratory Regulations
- Hazard Communication Standard
- Laboratory Standard
- Bloodborne Pathogen Standard (universal precautions)

NIEHS Required Programs (in compliance with OSHA regulations)-PPE
- Eye/Face Protection
- Respiratory Protection
- Hearing Protection
- Foot Protection
- Hand Protection

Other Training
- Ergonomics (Repetitive Motion)
- Back Injury Prevention
- Fire Extinguisher Training
- First Aid/CPR/AED Training

Personnel exposure to chemical, physical and environmental hazards are assessed by the NIEHS HSB. The NIEHS HSB recommends appropriate controls/PPE based on the hazards in the animal facility.

Potential chemical hazards include waste anesthetic gases (Isoflurane), cleaning materials and cage wash process chemicals. Sampling results are compared to appropriate occupational exposure limits, control measures (engineering, administrative, PPE) are identified and evaluated for effectiveness and results of hazard assessments are communicated to the employees.

Potential physical hazards include; hazardous noise, ergonomic, and dust. These hazards are assessed and compared to appropriate occupational exposure limits, control measures (engineering, administrative, PPE) are identified and evaluated for effectiveness and results of hazard assessments are communicated to the employees.

Physical and chemical hazards are assessed on both a routine (at least annual isoflurane assessments to verify effectiveness of procedures and scavenging system) and as operations change (introduction of new noise producing equipment, personal ergonomic concerns).

The safety protocol review and approval process outlines the containment procedures and the appropriate PPE to use when handling contaminated animals and waste materials to reduce potential for injuries.

Environmental measurements (heat and humidity) are collected periodically in the cage wash areas to assess any changes in environmental conditions. Workplace air samples for evaluating employee exposures to wood dust and animal feed particulates during automated dispensing operations in the clean and dirty cage wash areas are performed as necessary. Surveys are conducted in noise generating areas of the animal facility and engineering controls and adjustments recommended, if needed, in areas where noise levels approach or exceed acceptable levels or duration. Employees are required to wear protective hearing devices when working in the cage wash areas. These employees undergo annual audiometric exams as required by the NIEHS and the contractor's hearing conservation programs. The results of the most recent noise survey completed in June 2014 confirmed that levels in both Redacted by agreement areas were below the OSHA permissible exposure limit of 90 dBA for an eight hour period.

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

The HSB provides for the annual certification of Biological Safety Cabinets through contract services and performs in-house annual ventilation testing of chemical fume hoods. CMB is responsible for ensuring the annual recertification of cage changing/animal transfer stations, bedding disposal systems and IVC air handlers.

Safety showers are inspected on an annual basis by the staff of the Facilities Management Branch. Eyewash stations are activated on a weekly basis by husbandry personnel to verify operation and that flushing fluid is available.

3) Describe situations where respiratory protective equipment is available or required, such as cage washing facilities, feedmills, etc. Describe how such equipment is selected and how respirator fit testing and training in the proper use and maintenance of the respirator is provided.

Respiratory Protection training is included as part of the Laboratory Health and Safety and Introduction to Health and Safety at NIEHS training courses.
It is the policy of NIEHS to protect all employees from exposure to airborne radioactive, chemical, or biological contamination by engineering or administrative controls. If necessary, these may be supplemented with appropriate respiratory protection. NIEHS has a written Respiratory Protection Program that meets the OSHA 29 CFR 1910.134 Respiratory Protection Standard. This program includes initial selection criteria, medical evaluation, qualitative and quantitative fit testing, training of workers and annual maintenance checks by the HSB. FEFA also has a Respiratory Protection Program that meets this standard for contract employees. Air purifying respirators with combination cartridges are used as stipulated by an approved safety protocol. Powered Air Purifying Respirators (PAPR) are used by a small number of employees that have allergy problems. Respirators (e.g, N-95) are used in hazardous areas and soiled cage wash.

4) Describe program policies to ensure personnel safety when working with rack/cage washers, other sanitation/sterilization equipment, and other heavy equipment such as scrapers, tractors, and farm machinery. Describe the training program that supports these policies.

The HSB has evaluated the safety of walk-in rack washers located in Redacted by agreement areas and the bulk autoclave located in the ABF building. The rack washers contain an easily accessible emergency shutoff mechanism which de-energizes the washer and allows the cagewash door to be opened from the inside. There are also emergency shutoff mechanisms located outside the machine on both the clean and return side of cage wash. This is a lockout system whereby the emergency stop mechanism, once activated, must be actively disengaged before the machine can be restarted. Proper instructional signage is posted and cagewash operators are thoroughly trained on all safety requirements.

Redacted by agreement

The operator is instructed to always make a thorough visual check of the interior of the autoclave before closing this door. There is an emergency shutoff switch on the power box outside the machine that immediately terminates the sterilization cycle and allows opening of the door. Proper instructional signage is posted and the autoclave operators are thoroughly trained on all safety requirements.

The contractor conducts its own training programs and provides PPE for employees using the cage/rack washers, autoclaves, and other sterilization equipment, the automated Ducon system for the transport of animal feed and bedding, and other heavy machinery or equipment.

A fork lift is used in the ABF food and bedding area to move pallets. An accredited outside vendor provides forklift training. The certification class consists of a power point presentation followed by a written test and proficiency demonstration. Drivers are recertified every three years. Drivers are taught to use the horn when preparing to back up. Removing the foot from the pedal will automatically stop the forklift.

The items of personal protective equipment may include, but are not limited to respiratory protection equipment, gloves, clothing, eye/face protection, hearing protection and slip resistant safety shoes. The HSB reviews the practices in the use of PPE to ensure they are adequate.

Emergency eyewashes and safety showers are provided in areas in the animal facility where potential splash hazards to corrosives, eye irritants, or chemicals that are toxic via skin and/or eye contact exist.

vii. Medical Evaluation and Preventive Medicine for Personnel [Guide, pp. 22-23]

1) Identify the individual(s) and/or office responsible for developing and monitoring the medical evaluation and preventive medicine program.

Occupational health services are administered by the HSB. Examinations are conducted in the NIEHS Health Unit which is staffed by a full time nurse and a physician with regular clinic hours.

The CMB Animal Husbandry Support contractor has an occupational health program that is comparable to the NIEHS program. Occupational health services for FEFA employees are provided by the Duke Occupational Health Program.

2) Describe the categories of personnel (research staff, visiting scientists, animal care staff, students, support staff, etc.) included in the program.

All NIEHS personnel participating in research projects involving animals are required to enroll and be cleared to work with animals through the NIEHS occupational health surveillance programs. All contract animal care staff must enroll and be cleared through the contractor's occupational health surveillance program. Individuals who are not employed by NIEHS (i.e. special volunteers, summer students) must ensure that their personal physician completes an individual health and job related risk assessment and determines that the individual can safely work with animals. Validation of this determination must be provided to the NIEHS health clinic.

Non-affiliated members and non-scientific members of the ACUC are offered enrollment in the NIEHS occupational health program commensurate with the risk of animal exposure. These health monitoring services are provided at no cost to ACUC members.

3) Describe general features of the medical evaluation and preventive medicine programs, including pre-employment/pre-assignment health evaluation, periodic medical evaluations, immunization programs, and procedures for communicating health related issues.

Examinations include an initial examination at employment and periodic examinations on an eighteen to twenty-four-month schedule. Examinations are tailored to the work requirements and potential exposures of the employee but include the following at a minimum:

- Comprehensive medical/personal habits history
- Occupational exposure history
- Complete physical examination/vital signs
- ECG (baseline)
- Vision examination
- Audiometric examination, as indicated
- Appropriate vaccinations, based on potential occupational exposures (e.g. Hepatitis B for work with human blood, fluids, or tissues; tetanus if not current for personnel working with animals)
- Pulmonary function, as indicated
- Chest x ray, as indicated
- Blood chemistry, CBC, serology, urinalysis
- Special diagnostic tests as indicated from medical/occupational histories
- Animal allergy questionnaire and immunologic testing for personnel working with animals

4) Describe special precautions or procedures for personnel exposed to potentially hazardous species (nonhuman primates, sheep, etc.) or agents (infectious agents, human origin tissues, chemicals/toxins, etc.).

The safety protocol defines the precautions and procedures that are applicable for the hazardous agents involved in a study and includes the containment devices, PPE (e.g., glove material, respirators, etc.) and work practices required for personnel participating in the study.

The occupational health physician reviews the employee's potential exposures as well as pertinent medical and occupational histories. Based on this information, additional surveillance measures and biological indicators of exposure may be initiated.

The NIEHS medical evaluation and preventive medicine program also ensures the employee has appropriate and up-to-date immunizations (e.g., tetanus, HBV) and can safely wear respiratory protection if the safety protocol stipulates such use. During the medical examination, employees are counseled on effective means of preventing laboratory animal allergies. The Occupational Health Unit,

HSB developed a brochure on "Allergies in the Research Laboratory" as an aid in conveying this information to research personnel.

As part of their initial health and safety training, employees are instructed to report observed hazards, exposure incidents (suspected or overt), and all accidents including animal bites, scratches, or allergic reactions to their supervisor and the HSB. Post-exposure medical surveillance is initiated depending on the nature and possible severity of the incident.

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

The DDIR/IO has a policy memo: "Communicating Animal Care and Use Concerns within the NIH Intramural Research Program" that is posted on the OACU website. This memo strongly encourages anyone with an animal welfare concern to communicate that concern and provides a range of individuals they can approach. The memo provides the OACU contact information as an avenue for anonymous reporting and the OACU voicemail phone tree will direct callers to the Director's voicemail to allow messages to be left outside normal duty hours. This memo has been sent to all employees and is prominently posted in the animal facility and common areas in Redacted by agreement

Additionally, procedures for reporting animal welfare concerns are included in the NIEHS Investigator Training Course, "Humane Care and Use of Animals in Research" and in the Triennial refresher training course required. The NIEHS Guidelines for Resolving Non-Complaince Issues is available on line. The "Animal Concerns Contact List" is prominently posted in the vivarium, laboratory areas, and common areas in Redacted by agreement and on the CMB website. Concerns can be emailed directly to AnimalConcerns@niehs.nih.gov, anonymously via http://apps.niehs.nih.gov/animalconcerns, or via the NIEHS Junction (make a suggestion or report a concern). Protection against reprisal is included in all training sessions.

The facility manager or his ARS designee reviews procedures for reporting animal welfare concerns with security, maintenance, and custodial staff during training sessions. All summer students receive this information during orientation, regardless if their summer work involves animal contact. This information is reiterated to summer students working with animals in the NIEHS Investigator Training Course, "Humane Care and Use of Animals in Research," which they are required to attend.

CMB investigates any animal concern which may include evaluation of the animals, ASP review, and speaking with involved individuals including animal technicians, laboratory technicians, and the investigator. A report of the incident and findings are made directly to the ACUC Chair and Attending Veterinarian. The Chair may appoint a subcommittee to further investigate or may call a convened meeting of the ACUC. The ACUC determines the appropriate course of action based on the incident involved. Documentation of the issue describing findings, and required corrective actions if applicable is maintained. Documentation of reportable incidents are sent to the ACUC Chair, APD, PI, Laboratory/Branch Chief, and Scientific Director. This information is also communicated to the concerned employee, unless anonymous means were used for reporting.

Instances of noncompliance (both protocol and animal program related) are likewise reported to the IO through the OACU Director, in a timely manner, to effect appropriate Institutional communications. When issues are determined to represent serious or continuing non-compliance the Director, OACU provides a preliminary report to the DDIR/IO and to the Office of Laboratory Animal Welfare (OLAW). After the ACUC investigation has occurred and corrective actions have been approved by the ACUC, a comprehensive, final report is submitted to the DDIR/IO via the Director, OACU. The DDIR/IO submits a final report to OLAW. After OLAW acknowledges that the actions taken are sufficient, the incident is presented to the ARAC members for their information/education.

When an ACUC suspension occurs, it is reported to the DDIR/IO through the Director, OACU who will provide an initial preliminary report to OLAW. When the ACUC suspends an ASP, the Scientific Director in consultation with the ACUC, shall review the reasons for the suspension, and the appropriate corrective action(s). The ACUC will submit a final report describing the incident and the corrective actions through the Director OACU to the DDIR/IO. The DDIR/`IO will review the reasons for suspension, and will review and approve or request changes to the corrective action(s) taken, and will make a full report to OLAW.

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 an appendix.

i. Describe Committee membership appointment procedures.

The NIEHS Scientific Director, through delegated authority from the IO/DDIR, appoints the ACUC members, Chairperson, and Deputy Chairperson. Appointments are generally for a three-year term and reappointments are possible. Scientific members represent the scientific programs using animals at NIEHS. Every effort is made to fairly represent the major Laboratory/Branches using animals. No more than 3 members are appointed from any one branch.

ii. Describe frequency of Committee meetings.

The NIEHS ACUC meets once a month.

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

New ACUC members are given a formal orientation to the NIEHS animal care and use program by the ACUC program coordinator. This training covers relevant legislation, regulations, ACUC policies and procedures, and ACUC member responsibilities. A PowerPoint presentation has been developed for this purpose, is updated on a regular basis, and meets all NIH requirements for ACUC member

training. New members are provided with extensive NIEHS ACUC reference documents and those members not already familiar with the animal facility are given a tour of the facility.

New ACUC members are not assigned as primary reviewers until they have attended several ACUC meetings to become familiar with the review process. Once assigned as a primary reviewer, the new member works closely with a member of the ACUC office staff to assist with the initial review procedures. An ASP Review Checklist is used by all ACUC members to ensure a thorough, consistent, well documented review.

As mentioned previously all ACUC members also complete the NIEHS course on "Humane Care and Use of Animals in Research" and the on- line triennial refresher course.

Committee members are encouraged and supported financially to enhance their understanding of animal care and use in science by attendance at ACUC-related training opportunities such as those sponsored by Public Responsibility in Medicine and Research (PRIM&R), the North Carolina Association for Biomedical Research (NCABR), and OLAW sponsored courses such as "IACUC 101" and "ACUC Advanced." Webinars, such as those presented by OLAW, are available at NIEHS and are frequently attended by ACUC members.

ACUC members are kept up to date concerning relevant animal care and use issues through forwarded postings, published articles, and online newsletters such as Animal Research News and Analysis, NCABR Media Scan, and the AMP News Service.

b. Protocol Review [Guide, pp. 25-26]

A blank copy of your institution's protocol review form should be provided as an appendix. Also include forms used for annual renewal, modifications, amendments, etc., as applicable.

i. Describe the process for reviewing and approving animal study protocols, including research and teaching proposals. Include a description of how animal study protocols that do not involve a formal grant proposal are reviewed and approved (i.e., pilot studies or internally funded studies). Include a description of how the IACUC/OB weighs the potential adverse effects of the study against the potential benefits that may result from the research. Describe how protocols that have the potential to cause pain or distress to animals are reviewed, alternative methodologies reviewed, veterinary input solicited, and studies controlled or overseen. Specify how animals and experimental group sizes are justified.

Protocol Review Procedures:

There are two valid methods of ACUC review allowed by the PHS Policy (1) full-committee review by a convened quorum of the members of the ACUC, or (2) designated member review by one or more members. Guidance on what constitutes a significant change and therefore warrants review and approval via full ACUC review, veterinary verification, or other administrative review is provided for the IC ACUCs through the ARAC Guideline Regarding Significant Changes to Animal Study

Proposals and further delineated through the NIEHS Animal Care and Use Committee Guideline.

Note: An administrative process is in place to review the following minor changes:

- Addition of trained personnel (change in PI requires new ASP and full committee review). This is documented in the meeting agenda and minutes.
- Increase in animal numbers of ≤ 10% (based on number approved in original ASP). This is documented in the meeting agenda and minutes.
- Correction of typographical errors, grammar and contact updates
- Change in animal use location outside the animal facility. The change is captured in the semiannual laboratory site visit documentation and reflected in the ASP by the ACUC Office.

1. Standard Full Committee Review (FCR):

This method is used for ASP's and amendments that describe the following significant changes

- Novel concepts/Change in Study Objectives
- Changes which may involve an increase in potential pain/distress to animals
- Change in species
- Column E studies
- Survival surgery
- Prolonged restraint (>20 minutes)
- Exceptions to the Guide
- Hazardous Agent or drug usage for which the PI has no documented experience at NIEHS
- Change in PI
- Procedures which raise concerns during pre-review.

Process: CMB veterinarians and the ACUC coordinator conduct a preliminary review of each proposal and amendment. A pre-review with questions and requests for clarification is sent to the investigator. The proposal/amendment is assigned to one committee member for primary review and presentation to the committee. The primary reviewer should contact the investigator with any additional questions/concerns prior to the ACUC meeting. Revisions must be received 6 business days before the meeting. Revisions of each proposal/amendment are distributed to ACUC members for their review at least 3 business days prior to the convened meeting. Each primary reviewer completes an "ASP Review Checklist" documenting review of the proposal and facilitating presentation at the convened committee meeting. After discussion led by the primary reviewer each proposal is brought to a vote. The proposal may be approved, not approved- requires modifications to secure approval, or approval withheld.

If not approved- requires modifications to secure approval, no work may be initiated on the proposal. As approved by the full committee at the December 2013 ACUC meeting and subsequently reviewed and approved in writing annually (January 2017) by all current ACUC members, the quorum of members present at a convened meeting may decide by unanimous vote to use Designated Member Review (DMR) subsequent to FCR when modification is needed to secure approval. However, any member of the ACUC may, at any time, request to see the revised protocol and/or request FCR of the protocol. If DMR is approved and FCR is not called, the date that

the designated reviewer approves the ASP/amendment (documented on the ASP Review Checklist) is the approval date.

If approval is withheld no work may be initiated on the proposal and it must come back to the full committee for final review and approval. The ACUC meeting date at which a proposal is approved is considered the date of approval.

2. Designated Member Review (DMR)

Designated Member Review is used for ASPs and amendments which are outside of the scope of those requiring FCR as described above.

Process: CMB veterinarians and the ACUC coordinator conduct a preliminary review of each proposal or amendment. A pre-review with questions and requests for clarification is sent to the investigator and the designated member reviewer, who is selected based upon expertise in the subject matter, whenever possible, and assigned by the ACUC Chair. ACUC members are provided with the ASP number, title, PI name, and a brief description of the proposal via electronic correspondence weekly and are asked to respond within 24 hours if they wish for the submission to be reviewed by FCR. The full ASP/amendment is available to all committee members, upon request. If full committee review is not requested the designated member has the authority to approve, require modification to secure approval, or refer to the ACUC for full committee review. Actions taken on any designated member review are reflected in the ACUC meeting agenda and minutes. The date that the designated reviewer approves the ASP/amendment (documented on the ASP Review Checklist) is the approval date.

Examples that can be reviewed by Designated Member Review:

Animal Study Proposals:

- ASP's that involve only invertebrates
- Breeding and production only
- Tissue collection

Amendments:

- Change involving only invertebrates.
- Change in animal genus/species/stock/strain with no expected adverse phenotypes.
- Husbandry issues
  - changes in feed or water that is part of scientific portion of study
  - changes in type of caging or bedding that is part of scientific portion of study

  Note: The use of metabolism cages for longer than 24 hours, which represents an exception to the Guide, is a significant change requiring full committee review.

- Feed restriction < 24 hours in duration (unless it potentially impacts the health or wellbeing of the animal)
- Addition of non-invasive procedures such as breeding/backcrossing/behavioral phenotyping/imaging
- Addition of single housing
- Change in euthanasia or anesthesia/analgesia agent/method (if in compliance with regulations/policies)
- The following UNLESS it has the potential to impact on health or well-being of the animal:

- Change in blood collection site or volume
- Change in number or frequency of sampling timepoints
- Change in route, concentration, or volume of compound administration
- Change in vehicle of compound administration
- Change in dosage or dosing frequency

- Transfer of animals to an offsite collaborator who is AAALAC accredited and OLAW assured while maintaining ownership of the animals post-transfer. (requires a copy of the approved protocol from the receiving institution)
- Changes which may involve a decrease in potential pain/distress to animals
- Increase in animal numbers of >10% (based on the number approved in the original ASP)

In any of these examples, if the veterinary preliminary review indicates any significant issues, potential impact on animal health or well-being, or if there is any question about the significance of the change, it will be referred to full committee for review.

PROTOCOL PROCESSING PROCEDURES

1. **Standard Review:**
ASPs and amendments are submitted to the ACUC Office and undergo a pre-review by the ACUC staff and CMB veterinarians. Materials submitted by the first of the month, will be reviewed at that month's ACUC meeting. Materials for review at an ACUC meeting are distributed to committee members at least 3 business days prior to the meeting. Investigators are notified in writing of the outcome of review of their proposals.

2. **Expedited Review:**
On rare occasions, i.e., when strong scientific need, limited availability of animals or facilities requires a rapid response, an expedited review of an ASP or amendment normally requiring FCR per our committee's SOP's may be requested. These expedited reviews are handled following our DMR process.

Process: The proposal/amendment is submitted with an expedite request including the scientific justification for the request. The submission is reviewed by the ACUC coordinator, a CMB veterinarian, and the ACUC Chair with emphasis placed on the scientific need for the requested expedited review. The Chair determines whether an expedited review is warranted. The ASP/amendment is distributed to all ACUC members with a request for expedited review including the justification for the request. Committee members are given 24 hours to review and call for full committee review, if desired. Response from a committee quorum is assured and documented. If any committee member requests full committee review, the expedited process is stopped and the proposal/amendment is sent to the full committee for review at a convened meeting. If full committee review is not requested, the ACUC Chair designates a member to review the proposed research project with authority to approve, require modifications to secure approval, or refer to the ACUC for full committee review. Actions taken on any expedited review are reflected in the ACUC meeting minutes. The date that the designated reviewer approves the ASP/amendment (documented on the ASP Review Checklist) is the approval date.

Areas of emphasis during protocol review:

Animal numbers and experimental group sizes are described in the ASP and statistically justified when possible. A biostatistician serves as a member of the ACUC and assists investigators with determining the appropriate group size necessary to obtain valid results.

The ACUC carefully considers the harm a study could impose upon an animal and works diligently to mitigate it, when possible. The subject experts (lab/branch chiefs) must sign off on the scientific merit of the proposed work, which validates the studies potential for benefit(s). Studies with the potential to cause more than momentary or slight pain and distress to the animals require a documented search for alternatives to potentially painful procedures. Guidance on conducting a search for alternatives is provided to all investigators, and an NIEHS librarian is available to assist with or complete the searches. The ASP form requires that the PI specifically address these issues. Discussion between the PI, CMB veterinary staff, and the ACUC primary reviewer occurs during the pre-review process. Further discussion and veterinary input occurs during the ACUC meeting review. In cases when the ACUC stipulates veterinary oversight or monitoring of a study, the VMS provides feedback in the form of a Memo to the Record (MTR) to the ACUC office that is maintained electronically and the information is presented to the ACUC at the monthly meetings.

Also note, review and approval of renewals adheres to the standards referred to in the ARAC Guideline for Review, Approval, & Post Approval Monitoring of Animal Study Proposals Including Designated Member Review.

ii. Describe process for reviewing and approving amendments, modifications, and revised protocols. If applicable, include a description of "major" vs. "minor" amendments.

The process for reviewing and approving amendments, modifications, and revised protocols is described above.

Also note, review and approval of amendments adheres to the standards referred to in the ARAC Guideline for Review, Approval, & Post Approval Monitoring of Animal Study Proposals Including Designated Member Review.

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

i. Experimental and Humane Endpoints [Guide, pp. 27-28]
Describe how criteria for determining alternatives to experimental (humane) endpoints are developed, approved, and applied. Include a description of monitoring systems in place for studies for which information on alternative endpoints are not available.

During the planning and the veterinary pre-review of the ASP/amendment, the PI consults with CMB veterinarians to develop reliable humane endpoints and a monitoring plan with assessment criteria and the appropriate response when endpoints are reached. The PI outlines the agreed upon monitoring plan and procedures, the

scientifically sound and humane study endpoint, and the trained study participants in the ASP/amendment.

In addition, PI's are required to consider alternatives to potentially painful procedures in animals. The NIEHS library has staff dedicated to performing literature searches for alternatives. Librarians are provided a copy of the ASP and meet with the PI prior to initiating a search. The PI reviews the results and conclusions are incorporated into the ASP.

Prior to working with animals, a pre-study meeting is required for all new studies as well as repeat studies where complications, staff changes, or new endpoints have been approved by the ACUC. This meeting is attended by investigative staff, VMS staff, the animal facility manager, and animal husbandry technicians and outlines the experimental design and endpoints, humane endpoints, supportive husbandry procedures, and safety requirements.

The NIEHS program follows the ARAC Guidelines for Endpoints in Animal Study Proposals. Monitoring parameters for appropriate endpoint determinations include body weight/reduced rate of weight gain, food consumption, body temperature alterations, tumor burden, and appearance, and response to treatment (i.e. ulcerative dermatitis). Documentation of these parameters is required.

Trained animal technicians monitor animals daily and report clinical concerns to the VMS. For studies where clinical outcome and/or the humane endpoints are unknown, the ACUC requires that VMS monitor these studies, assist with defining optimal supportive measures, and review required documentation (i.e. body weight logs). VMS summarizes clinical observations via a MTR and summaries are presented at the monthly ACUC meetings. Copies are maintained by the ACUC office. Development of an unanticipated clinical condition is documented by VMS and reported to the ACUC. In consultation with a CMB veterinarian, treatment/supportive measures or intervention strategies are discussed and endpoints are developed relating to this unanticipated clinical condition.

If an unanticipated clinical condition results in a change to the pain or distress category, an amendment is required describing the clinical condition, treatment/supportive measures/intervention strategies, humane endpoints and monitoring refinements, reclassification of the pain and distress category and alternative justification submission.

When clinical outcome and humane endpoints are unknown, the ACUC may also require the completion of a pilot study or range-finding study with close oversight by VMS and documentation of the study.

ii. Unexpected Outcomes that Affect Animal Well-being [Guide, pp. 28-29]
Describe how unexpected outcomes of experimental procedures (e.g., unanticipated phenotypes in Genetically Modified Animals) are identified, interpreted, and reported to the IACUC/OB.

Unexpected outcomes, including unanticipated phenotypes in genetically modified animals, may be identified by trained animal husbandry and breeding technicians,

VMS personnel, CMB veterinarians, or investigative staff. Animal technicians monitor animals daily and report clinical concerns to the VMS. Clinical issues that potentially impact animal well-being or require more extensive monitoring would necessitate a discussion between the investigator and a CMB veterinarian. Treatment/supportive measures or intervention strategies and endpoints relating to the clinical condition are formulated through this discussion, summarized by the VMS in an MTR, reported to the ACUC, and then maintained by the ACUC office. If the clinical condition changes the pain and distress category of the animal, an amendment is submitted for ACUC review which describes the clinical condition, treatment/supportive measures/intervention strategies, humane endpoints and monitoring refinements, reclassification of the pain and distress category, and alternative justification submission.

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.

Physical restraint is the use of manual or mechanical means to limit some or all an animal's normal movement for such purposes as examination, collection of samples, drug administration, or experimental manipulation. Prolonged restraint is defined as physical restraint of a conscious animal in a normal postural position lasting longer than 20 minutes or restraining an animal in an unnatural position (i.e. dorsal recumbency) beyond the minimum needed for examination/sample collection/ drug administration. In general, prolonged restraint requires acclimation which encompasses at least 3-5 repeated exposures to the same conditions, while holding the exposure time to these conditions constant. Prolonged restraint is avoided unless it is essential for achieving research objectives and is specifically approved by the ACUC.

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.

Ultrasound platform and gauze restraints are used for less than 5 minutes to perform ECG imaging in conscious mice restrained in dorsal recumbence. Acclimation for this procedure is not performed; it has been determined that acclimation to this procedure is not possible. Animals are continuously monitored during the procedure.

Mice are held in conical tubes up to 3 hours once daily for 5 days to trigger the neural mechanisms that create stress-induced feeding behavior. Scientific justification is provided in the ASP and has been approved by the ACUC. The ACUC requested VMS to observe the first five animals exposed to this restraint to assure acclimation was possible and to provide training to research staff in

identifying observation parameters. Trained research staff continuously monitor mice during the restraint period. If an animal fails to acclimate as determined by 15 minutes of persistent struggling behavior, the animal is removed from the restraint study. Acclimation is not performed as it would interfere with the model.

For all studies, veterinary care is provided if lesions, illnesses, or abnormal behaviors occur.

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 institutional policy(ies) regarding multiple survival surgery (major or minor) on a single animal.

Multiple survival surgery on a single animal is discouraged and can be approved only when (a) the surgical procedures are essential components of a single research project, (b) are justified for scientific reasons by the PI, or (c) required as routine veterinary procedures or to protect the health and well-being of the animal as determined by the veterinarian.

2) Describe the procedure for approving multiple survival surgery (major or minor) and the criteria used to determine the potential impact on the animals' well-being.

ASPs or amendments involving multiple survival surgery are critically reviewed by the ACUC by Full Committee Review. Details of the surgery are required, along with identification of the person performing the surgery, their qualifications, and where the surgery will be performed. Only procedures which are essential components of a single study and which are controlled for adequate post-operative recovery time are approved. The PI must provide scientific justification for the procedures, assurance that alternative procedures are not available, and the methods and sources used to make this determination.

ASPs or amendments that involve the purchase of commercially-available-surgically-altered rodents are also given consideration by the ACUC as models of multiple survival surgery. Adequate recovery time is ensured between the commercial and in-house procedures.

3) Summarize the protocols currently approved that involve multiple major survival surgical procedures and the time allowed between procedures on the same animal. Describe the method of institutional monitoring.

Eight ASPs involve multiple survival surgical procedures Redacted by agreement Redacted by agreement at NIEHS. All survival surgeries are performed in the NIEHS animal facility.

- Mice undergo intracranial injection of AAV and 3 weeks post-operatively undergo electrode implantation surgery Redacted by agreement

- Mice undergo intracranial injection of AAV and 4 weeks post-operatively undergo surgical electrode/optical probe implantation. Redacted by agreement Redacted by agreement
- Mice undergo ovariectomy followed two weeks later by surgical decidualization Redacted by agreement
- Intracranial injection in rats followed 4 weeks later by IC cell transplantation Redacted by agreement
- Mice undergo intracranial injection of AAV and 2 weeks post-operatively undergo electrode implantation surgery. Redacted by agreement

Two ASPs Redacted by agreement involve the purchase of commercially available surgically-altered mice followed by a second surgical procedure at NIEHS. Adequate recovery time is allowed between the two procedures.

- Mice undergo ovariectomy/castration at the vendor followed by IP minipump implantation 14 days later Redacted by agreement
- Mice are vendor vasectomized followed 2 weeks later by surgical decidualization.

VMS and study participants closely monitor animals following all surgical procedures.

v. Food and Fluid Regulation [Guide, pp. 30-31]

1) Describe experimental situations that require food and/or fluid regulation. Note: This does not include pre-surgical fast. List title of the experiment(s), justification, species involved, and length and type of food/fluid regulation.

Physiological Function of Glis Proteins Redacted by agreement Food is withheld from mice for 16 hours prior to blood collection for glucose and insulin testing. Water is available at all times.

Sir2 and Nuclear Receptors in Aging and Age-associated Diseases, Redacted by agreement Food is withheld from mice for 16 – 24 hours prior to blood collection for glucose and insulin testing. Water is available at all times.

In Vivo Electrophysiological and Behavioral Analyses of CA2 Function Redacted by agreement Redacted by agreement Food intake is restricted by 15% of daily *ad libitum* amount in rats to motivate exploration of a novel environment during electrophysiology testing. Water is available at all times.

Functional Assessment of Hippocampal Area CA2 Redacted by agreement Food intake is restricted by 15% of daily *ad libitum* amount in rats to motivate exploration of a novel environment during electrophysiology testing. Water is available at all times.

Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders [Redacted by agreement] Food intake is restricted by 15% of daily *ad libitum* amount in rats to motivate exploration of a novel environment during electrophysiology testing. Water is available at all times.

Use of transgenic mice to study noradrenergic neuron development, diversity, and function [Redacted by agreement] Food intake is restricted by 15% of daily *ad libitum* amount in rats to motivate exploration of a novel environment during electrophysiology testing. Water is available at all times.

Sir2 and Nuclear Receptors in Aging and Age-associated Diseases, [Redacted by agreement] Food intake is restricted by 30% of daily *ad libitum* amount for the lifespan of mice to study positive effects on lifespan and age-related diseases. Water is available at all times.

Developmental Pharmocogenetics [Redacted by agreement] Food is withheld from mice 36-48 hour fast to study the molecular mechanisms of a receptor's regulation of hepatic physiology in response to fasting.

Glucocorticoid Receptor Phosphorylation Site Knock-in Mice [Redacted by agreement] Food is withheld from mice 36-48 hour fast to address the effects of prolonged glucocorticoid signaling. Water is available at all times.

2) Describe animal health monitoring procedures and frequency (e.g., body weight, blood urea nitrogen, urine/fecal output, food/fluid consumed).

Animal care personnel monitor animals daily. Monitoring frequency, parameters that must be documented (i.e. body weights, food intake), and clinical changes and pre-determined quantitative limits (i.e. percentage body weight loss or lack of gain) requiring study termination, are outlined in the ASP and followed by study participants and VMS. Monitoring records are maintained at the room level and are available for evaluation by the VMS. For food restriction studies, body weights are recorded at least weekly.

3) Describe methods of ensuring adequate nutrition and hydration during the regulated period.

The NIEHS program follows the basic standards set in the ARAC Guidelines for Diet Control in Laboratory Animals and has developed refinements in the NIEHS Guidelines for Food Regulation in Rodents.

All feed and caloric restriction studies allow free access to water. As stated above, animal care personnel monitor animals daily and for long term caloric restriction VMS assists in animal health monitoring.

vi. Use of Non-Pharmaceutical-Grade Drugs and Other Substances [Guide, p. 31] Describe the rationale and consideration given by the IACUC/OB for use of non-pharmaceutical grade drugs or other substances, if applicable.

Pharmaceutical grade compounds are used whenever possible in laboratory animals. NIEHS follows the ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

The use of non-pharmaceutical grade materials must be described and justified in the ASP and approved by the ACUC. Consideration is given to grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation compatibility and pharmacokinetics of the material to be administered as well as animal welfare and scientific issues relating to its use.

Use of non-pharmaceutical grade compounds that have been approved by the ACUC has been based on non-availability of pharmaceutical grade chemical, non-availability of pharmaceutical grade in size or concentration suitable for species being used or the scientific need to compare results with past experiments.

vii. Field Investigations [Guide, p. 32]
Describe special considerations used by the IACUC/OB when reviewing field investigations of animals (non-domesticated vertebrate species), if applicable.

N/A

viii. Agricultural Animals [Guide, pp. 32-33]
Describe considerations given and guiding documents used by the IACUC/OB when reviewing "biomedical" and "agricultural" research projects involving agricultural species as study animals, if applicable.

N/A

ix. Animal Reuse [Guide, p. 5]
Describe institutional policies and/or oversight of animal reuse (i.e., on multiple teaching or research protocols). Summarize the protocols currently approved that involve the reuse of individual animals.

Animals may be bred under one ASP and transferred to another ASP for experimental procedures. Animals are not reused in more than one experimental ASP.

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

a. Describe mechanisms for IACUC/OB review of ongoing studies and periodic reviews (e.g., annual review, 3-year renewals if PHS funded, etc.).

NIEHS ASP's are approved for a three-year period. At the end of the three-year period, an ASP may be resubmitted as a new proposal and will be reviewed in the same manner as the initial ASP. All ongoing proposals are reviewed annually. The PI reviews the proposal and completes an "Annual Review Form" indicating whether the study is to be discontinued, continued without changes, or continued with changes. If changes are required, an amendment is submitted for ACUC review. Annual reviews are reflected in the ACUC agenda and minutes.

b. Describe the process and frequency with which the Committee reviews the animal care and use program and conducts facility and laboratory inspections. Detail any criteria used for exempting or varying the frequency of reviewing satellite holding facilities and 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 an appendix.

Review of the NIEHS animal care and use program is an ongoing process. Issues are discussed at each ACUC meeting and members are informed about NIH wide animal research issues presented at the monthly NIH ARAC meetings.

The animal care and use program and facilities are formally reviewed semiannually using the Guide as a basis. As part of this process, ACUC committee members formally analyze and discuss the animal care and use program at a convened meeting, using the NIEHS AAALAC program description as a guide. ACUC members are assigned to each of five major topics for review: Institutional Policies, Veterinary Care, Laboratory Animal Husbandry and Physical Plant, Special Considerations, and Laboratory Visitation. They report to the full committee at a convened meeting identifying any changes, deficiencies requiring modification, and areas in which the program is working particularly well.

The committee also participates in a semiannual site visit of the NIEHS animal facilities which involves inspection of all animal holding and support areas, feed and bedding preparation areas, and inspection of vehicles used for transportation of animals.

Unannounced site visits of all laboratories where animals may be used are conducted semiannually by a subcommittee of the ACUC. Special attention is focused on areas where surgery (only non-survival surgeries permitted outside the vivarium) may be conducted. The surgical area and anesthesia and euthanasia equipment is observed. Animal monitoring and related procedures are discussed, appropriate transportation and cage/carcass disposal procedures are reviewed, handling, and use of controlled substances is examined, and availability of the approved ASP and understanding by all laboratory participants are assured. Participants are questioned about appropriate quality of procured animals and facility operations and any unexpected changes in experimental outcomes. Methods to minimize allergen exposure are also discussed as well as enrollment in the Occupational Health Program.

Written reports of semiannual reviews, signed by a majority of ACUC members, are sent to the NIEHS Scientific Director and the NIH Office of Animal Care and Use, who serve as delegates to the NIH Institutional Official. The most recent report is included as an appendix.

As described previously, contract personnel perform all animal husbandry and support functions in the NIEHS animal facility. Contract performance is assessed on an ongoing basis and a formal semiannual contract evaluation is conducted. In addition, the ACUC reviews this contract operation during the program review focused on animal husbandry and physical plant.

c. Describe institutional responses to deficiencies noted on regulatory inspection reports (e.g., government, regulatory agencies). Note: Copies of all such inspection reports for the past three years (if available) should be available for review by the site visitors.

The NIEHS is a federal facility and as such, it is not required to be registered with the USDA nor is it subject to routine USDA inspection. However, it is bound by the USDA regulations and submits an annual report to the NIH Office of Animal Care and Use for inclusion in a composite NIH Annual Report to USDA.

d. Describe other monitoring mechanisms or procedures used to facilitate ongoing protocol assessment and regulatory compliance.

Post approval monitoring is an ongoing process with various methods and levels of review. A pre-study meeting is required for complicated or novel studies that have been previously approved by the ACUC, prior to working with animals. This meeting is attended by investigative staff, VMS staff, the animal facility manager, and animal husbandry technicians and outlines the experimental design and endpoints, humane endpoints, supportive husbandry procedures, and safety requirements to ensure that all procedures and safety requirements are clearly understood. Most surgical and technical procedures are conducted by CMB staff or CMB provides training and oversight to investigative staff, assuring ongoing communication during conduct of studies. Animal care staff generate animal health reports based upon daily animal observations. VMS review these reports so that any unexpected events are quickly recognized. Facility inspections by CMB veterinarians and supervisory contract staff assess activities for protocol and regulatory compliance. The VMS is asked by the ACUC to monitor many studies and this is an ongoing process. Adverse phenotypes or unexpected experimental effects on animals are also reported and investigated by VMS. The VMS provides summaries at the convened ACUC meeting and submits a MTR to the ACUC office, which is filed electronically.

Additionally, semiannual reviews of the facilities and program; annual protocol reviews and OACU Observer participation serve as methods of post approval monitoring.

II. Animal Environment, Housing and Management

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

A. Animal Environment

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

a. Describe briefly the heating and air conditioning system performance. Provide method and frequency for assessing, monitoring, and documenting animal room or housing area temperature and humidity that is appropriate for each species. Note current (measured within the last 12 months), detailed (by room) performance data are to be provided as indicated on the enclosed Heating, Ventilation, and Air Conditioning (HVAC) System Summary appendix. If outdoor housing areas are used, so note.

The heating, ventilation, and air conditioning systems (HVAC) for the animal facility Redacted by agreement are separate from the rest of the facility. Each module is provided

with 100% outdoor air from a dedicated air handling unit (AHU). One hundred percent outdoor air is filtered and conditioned in 3 separate central air handling units for both supply and exhaust. In addition, each AHU is supported with a completely redundant stand-by unit for a total of 6 AHUs that serve the 3 animal modules. Key components of all supply and exhaust systems include fan arrays, pre-filters, pre-heat coils, cooling coils, humidifiers, ultraviolet lamps, HEPA filtration, and isolation dampers. Re-heat coils and humidifiers are located at each individual room to provide greater control of temperatures and humidity. All 100% outdoor air is HEPA filtered prior to entering the HVAC system. The Redacted by agreement Animal Facility cooling capacity is based in American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) extreme summer design conditions such that no excursions from set points occur.

Temperature and humidity sensors are in each animal room exhaust duct as well as in each animal room. Room temperatures and humidity are programmed and monitored on a twenty-four-hour basis through the Metasys Building Automation System (BAS). These records are maintained for a 5-year period. If either temperature or humidity exceeds the high or low limit set points an alarm condition will be indicated in the Building Engineers Redacted by agreement which is staffed 24 hours a day, and appropriate individuals are contacted. Any abnormalities are immediately checked and corrective actions are taken.

Temperature and humidity are also monitored at the animal room level with hi-lo room thermometers and recorded daily in the room log book.

b. If temperature set points and/or environmental conditions are outside the thermoneutral zone for the species, describe the process for ensuring behavioral thermoregulation (e.g., nesting material, shelter, etc.) and/or IACUC/OB approved exception.

Animal room temperature set points are 72 +/- 2° F for rodent rooms. This temperature is below the lowest mark for thermoneutral zone range in rodents (78.8-93.2° F) and meets the recommended dry-bulb macroenvironmental temperatures recommended in the Guide. Rodents are provided with bedding, nesting material, and/or a shelter to allow them to thermoregulate and avoid cold stress. Sources of supplemental heat are available for rodents requiring supportive care.

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

a. Briefly describe the performance aspects of the ventilation system. Provide method and frequency for assessing, monitoring, and documenting the animal room ventilation rates and pressure gradients (with adjacent areas). Note: current (measured within the last 12 months) detailed (by room) information is to be provided as indicated on the enclosed Heating, Ventilation, and Air Conditioning (HVAC) System Summary appendix.

Methods used to develop/confirm Metasys system readings for each animal room and all animal areas are a ductwork traverse method, where air flow velocity as well as duct size are used to calculate the cubic feet per minute. All rooms are calibrated at a minimum of once per year based on a preventive maintenance schedule or whenever there are questionable readings on the Metasys system reports. The Metasys system controls and monitors supply and exhaust air, thereby indirectly controlling and monitoring pressure gradients for all animal rooms. In addition, air pressure gradients between rooms and

clean and return corridors are monitored by smoke testing every two weeks and documented by animal husbandry personnel.

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

Ventilated cage racks are used that provide separate air flow for each cage. Air passes through a HEPA filter in a positive plenum of the supply unit. Filtered air then flows through the cage and is exhausted through a rough pre-filter, then a HEPA filter before being released into the animal room or the building exhaust system. Ventilated cage racks are monitored daily and are set to operate on emergency power. A visual alarm indicates any airflow parameters that are outside normal settings.

Air supply to the semi-rigid isolators is maintained on emergency power circuits, as well as battery back-up units that maintain air pressure for up to 20 hours. Each isolator is equipped with an air pressure alarm wired to the Metasys system for continuous monitoring.

The TSE Labmaster Metabolism Unit receives ambient air at a pre-set flow rate based on mouse size. The unit is set to operate on emergency power and is checked daily by the investigator when the system is in use. Redacted by agreement is notified regarding any issues related to the equipment including fluctuations of atmospheric gas concentrations that fall out of the acceptable, pre-determined range. Redacted by agreement also oversees the preventative maintenance contract.

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

Not applicable

3. Life Support Systems for Aquatic Species [Guide, pp. 84-87]
Provide a general description of institutional requirements for enclosures using water as the primary environmental medium for a species (e.g., aquatics). Describe overall system design, housing densities, and water treatment, maintenance, and quality assurance that are used to ensure species appropriateness. Please note that facility-specific tank design and parameter monitoring frequencies should be summarized in the Aquatic Systems Summary appendix.

Aquatic species are not maintained at NIEHS.

4. Noise and Vibration [Guide, pp. 49-50]
Describe facility design features and other methods used to control, reduce, or prevent excessive noise and vibration in the animal facility.

To the extent possible, activities that generate noise are conducted away from animal housing areas. Most animal rooms are in corridors away from cage wash areas. Most racks and carts are equipped with 8-inch phenolic casters and bumpers that help absorb vibration and noise when rolling. Casters are lubricated as needed to reduce noise. Radios or other sound generators are not used in animal rooms. To lessen human generated noise, the facility has two employee break rooms which are separate from and do not adjoin to any animal rooms; doors are kept closed at all times. Any personnel entering the animal facility are instructed to

minimize noise. Computers used in the animal rooms are programmed to shift into sleep mode after inactivity, thereby reducing any possible ultrasound noise. Equipment known to generate ultrasonic noise (i.e. tattoo equipment) is not routinely used in animal rooms.

To keep large equipment functioning at optimum levels, quarterly preventive maintenance inspections are performed on cage wash equipment by the Facilities Management Branch. In addition, emergency repairs are outsourced as needed. A major effort to minimize excessive noise in the animal facility involved installation of SounBreak Acoustical Panels (LabProducts, Inc) in all return corridors and in cage wash areas. These panels offer a 10-decibel reduction of reverberant noise. The fire alarm system in the animal facility uses a noise and frequency level that creates minimal to no recognizable auditory disturbance to rodents.

Attempts are made to keep vibration to a minimum in animal areas. Prior to any renovations or work involving vibration or noise generating equipment, sound measurements are made using a decibel meter. Duration of vibration/noise is also taken into consideration before proceeding. If equipment use is considered potentially detrimental to the animals, the animals are relocated to another area. Vibration absorbing mats have also been utilized under animal racks when necessary. Vibration from VCRs is minimized by having stand-alone air handlers. Proper function is routinely monitored and certified annually.

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

1. Primary Enclosures

Provide a description of primary enclosures used (e.g., cages (conventional, individually-ventilated cage systems (IVCS), etc.), pens, stalls, pastures, aviaries, tanks) in appendix.

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.

Recommendations contained in the current Guide are used to determine rodent space requirements. Guidance in cage size availability and housing density (including breeding animals) are provided to investigators (CMB SOP – Animal Caging Requirements; CMB SOP – Mouse Cage Capacity and Overcrowding). Overcrowded cages are reported to VMS via an Animal Health Report.

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

Exceptions to the Guide are reviewed and approved by the ACUC, either during ASP/amendment review or during review of relevant ACUC policies or CMB SOP's. Currently, there are no exceptions to the Guide space requirements.

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

a. 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, etc.).

Rodent cages have solid bottom flooring which is preferred by rodents. Rodents also make use of feeders and wire bar lids to exercise and express species-behaviors.

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).

All mice receive a combination of autoclaved Envirodri ™ and a Nestlet ™ to encourage complex nest building behavior. Sentinel mice also receive polycarbonate igloos with wheels.

Single and group housed rats receive autoclaved Envirodri ™ and polycarbonate shelters. Rat dams and pups receive autoclaved Envirodri ™ only as shelters may cause injury to the pups.

b. Social Environment [Guide, p. 64]

i. Describe institutional policy or strategy for social housing of social species.

General standards for environmental and social enrichment for all species are developed by an NIH subcommittee that includes Animal Program Directors, veterinarians, animal program managers, and enrichment specialists. These standards are further reviewed and approved by ARAC:
Guidelines for the Social Housing of Rodents and Aquatic Species

The NIEHS social housing program is further refined in the SOP "Social Housing for Mice and Rats" which has been approved by the ACUC and is reviewed triennially.

Rodents are housed in compatible groups to allow for social interaction and normal physiologic and behavioral needs unless single housing is required due to the following:

- Social Incompatibility – male mice may be single housed if required to alleviate or prevent fighting.
- Veterinary Concerns – veterinary staff may exempt an animal from social housing because of health or condition, or in consideration of its well-being.
- Scientific Necessity – written scientific justification for exception to social housing must be reviewed and approved by the ACUC. Note - scientific justification is not required for single housing of mice/rats in Buxco, LabMaster, or metabolism caging systems as pair/group housing would interfere with the scientific parameters under evaluation using these systems.

- Attrition/litter sexing - individual housing due to attrition of cage mates on study or when only one animal of a sex was produced from a litter.

ii. If social animals are not socially housed, provide justification, as approved by the IACUC/OB.

Single housing of animals may be required by an experimental protocol, such as for caloric restriction, measurement of food consumption, determination of activity levels, calorimetric measurements, infectious disease models, or as defined in the ACUC approved Social Housing for Mice and Rats SOP and must be reviewed and approved by the ACUC in an ASP.

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

Single-housed mice are provided both Envirodri ™ and Nestlet ™. Single-housed rats are provided Envirodri ™ and a shelter. Single housed animals have visual access to conspecifics.

c. 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.

Many of the experimental protocols involve routine handling of the experimental animals. These animals become acclimated to handling as a byproduct of the experiment. Consideration is given to maintaining consistence of husbandry and breeding staff to reduce the stress/fear response in the animals.

Many investigators habituate animals to novel procedures by gradually increasing exposure times to the environment or device prior to actual experimental manipulation as detailed in the ASP.

d. 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 environmental enrichment program is evaluated semi-annually by the ACUC during the program review and outlined in an SOP which has been approved by the ACUC and is reviewed triennially. New environment enrichment is regularly being evaluated for effectiveness and potential impact on research. Animal care personnel receive training in the behavioral biology of the species they work with and assist the veterinary staff in monitoring effects of enrichment in their daily care of animals.

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

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

N/A

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

N/A

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

N/A

f. Naturalistic Environments [Guide, p. 55]

i. Describe types of naturalistic environments (forests, islands) and how animals are monitored for animal well-being.

N/A

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

N/A

iii. Describe how animals are captured.

N/A

C. Animal Facility Management

1. Husbandry

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

i. List type and source of food stuffs.

Diet/ Description	Source(s)	Animal(s)	Shelf Life from Milling Date
NIH 31, Open Formula (autoclavable)	Envigo Madison, WI	Mice, Rats	6 months

LabDiet JL Mouse Breeder/Auto (5K20); (autoclavable)	LabDiet, Inc. (PMI) Richmond, IN	Mice (breeders)	6 months
Specialty Research Diets*; (e.g. Research Diets, Envigo)	Various vendors	Mice, Rats	6 months
Napa Nectar ™ Humane traps or for shipping; sterilized by manufacturer	SE Lab Group	Mice, Rats	18 months

* The type and source of the Specialty Research Diets varies with active research protocols.

ii. Describe storage facilities of vendors, noting temperature and vermin control measures. If more than one source, describe each.

a) LabDiet (Purina Mills, Inc. - PMI). LabDiet feeds are manufactured at a drug and synthetic estrogen-free plant at Richmond, Indiana. The plant is of metal construction with concrete floors. Temperatures range from 30-40ºF in the winter to 75-80ºF in the summer. The plant is site visited annually by NIH personnel (last visit July 2016), and their report forwarded to NIEHS. Pest control inspections conducted every other month are contracted out. All feed is stored on pallets until delivered directly by truck to our vendor-distributors, Granville Milling (Creedmoor, NC).

Granville Milling is the current contractor for supplying all LabDiet feeds (5K20). A truck delivers these feeds from PMI to the Granville Milling Warehouse in Creedmoor, NC and stored in their warehouse until delivered to NIEHS. The feed warehouse is comprised of two conjoining rooms measuring 32' x 26' x 8' and 32' x 24' x 8' and is climate controlled. It has 2" x 6" wood flooring, with plywood walls and ceiling, is air conditioned (<68ºF) and contains a dehumidifier to maintain relative humidity at about 55-60 %. All feed is stored on pallets. The bedding warehouse measures 65' x 35'. Ketch-all traps are maintained inside the facilities. The facilities are inspected daily for pests and to date none have been found. Traps and bait stations will be used around the outside perimeter of the storage building if evidence of wild rodents or other pests are observed. This facility was last site visited by Redacted by agreement in August, 2017.

b) Envigo, located in Madison, WI, is the current supplier for the NIH-31 autoclavable rodent diet. The facility is dedicated exclusively to the manufacture of diets for laboratory animals. The feed mill is constructed of concrete block with poured concrete floors. Feed is stored in an 18,000-square foot, steel building with poured concrete flooring. This facility is environmentally controlled with temperature and humidity monitored and recorded continuously (maintained at <70ºF, <55% RH). All diets are placed on new, disposable pallets. A comprehensive pest control program is in place. The facility is free of rodent, insect, and bird infestations. Daily in-house inspections of control devices are supplemented by weekly inspections, performed by an independent pest control service contractor. A truck delivers these diets from Madison, WI to customers or through one of 13 company-owned air conditioned distribution centers located throughout the US and Canada. NIH personnel visit the production site annually , and their report forwarded

to NIEHS. In May 2017, Redacted by agreement accompanied the NIH personnel on their annual site visit.

iii. Describe bulk food storage facilities, if applicable, noting temperature and vermin control measures. Note food storage areas within the specific animal facilities are described below in Section IV.B.4.a. Physical Plant.

Feed is stored in a 1284 sq. ft., climate controlled (55 to 65 ° F/50% RH) module within the Redacted by agreement warehouse on South Campus. The warehouse is a metal building with concrete slab and floor. Bulk shipments of feed are ordered every eight to ten weeks and the feed is stored on pallets which are placed on metal racks. At weekly intervals, small batches (two to four pallets) of feed are transported to the South Campus animal facility (ABF) where they are stored in Redacted by agreement an air-conditioned room (temperature 55 - 65°F), until autoclaved. After autoclaving, it is stored at least 24 hours to cool in Redacted by agreement at 55-65°F before it is transported manually or via the Ducon feed/bedding dispensing system to the dispensing sites for Redacted by agreement or to the dispensing site for Redacted by agreement Redacted by agreement Bags of feed that are not autoclaved are surface decontaminated by chlorine dioxide delivered in a misting tunnel located on the Redacted by agreement

The feed storage areas Redacted by agreement Redacted by agreement have insect monitoring stations and Ketch all live traps (with a hydration/nutrient source) that are checked daily by animal support staff.

Temperatures in Redacted by agreement the warehouse feed storage room and the warehouse bedding storage room are monitored on a 24-hour basis by the Metasys System.

iv. Describe food storage in animal rooms.

Feed is stored in the animal rooms in Rubbermaid bins. Feed cards are attached to the side of the bin and list feed type, chute #, mill date, date placed in room, and expiration date.

v. Describe food preparation areas.

Feed for the gnotobiotic isolators is packaged into transfer cylinders in Redacted by agreement Redacted by agreement for autoclaving.

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

Rodents: Feed is provided *ad libitum,* except when specified for a special study. Feeders are part of the rodent wire bar lid. Mash (ground feed mixed with RO/DI water) is used for veterinary care and support and is provided in a petri dish on the cage floor.

Studies requiring powdered diets may use round-glass containers with stainless steel tops, stainless steel box powdered diet feeders, or hanging stainless steel pendulum

feeders. The TSE Labmaster system uses hanging glass/stainless steel pendulum feeders for pelleted chow.

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

The QAL reviews the quality testing reports for each lot of standard NIH-31 rodent feed to confirm that the feed meets contract specifications for nutrient levels, and chemical contaminants. QAL performs testing for microbial contaminants. NIEHS receives a shipment of 1600, 25-lb bags every 8 weeks (approximately 7-8 shipments/year). Each lot of NIH-31 feed is analyzed for:

1) Microbial Quality: Coliforms, Total Plate Count (TPC), *Salmonella spp.*, *Pseudomonas spp.*, and *Citrobacter rodentium*.

2) Nutritional Quality: Protein, fat, fiber, phosphorous, calcium, carotene, Vitamin A, ash, selenium, and moisture levels.

3) Chemical Contaminants:

a) Pesticide screens
I PCB's
II organophosphates
III chlorinated pesticides

b) Heavy metals - arsenic, cadmium, lead, mercury, nitrates, and nitrite nitrogen

c) Mycotoxins: Aflatoxin B1, B2, G1, G2.

d) Nitrosamines

e) Butylated hydroxytoluene - BHT
Butlyated hydroxyanisole – BHA

f) Phytoestrogen content: Chemical assays

g) Estrogenic activity- Mouse bioassay/vaginal opening endpoints (if deemed necessary or requested by the investigator)

No NIH-31 feed is used until all testing has been completed and the feed approved by QAL for use.

All rodent feed (pallets or boxes) are labeled with the date the feed was received, milling date, and an expiration date. Stocks are rotated with the older shipment used before feeding the most recent shipment. A weekly inventory is performed on feed at all NIEHS storage locations. Therefore, the amount of feed in stock as well as mill dates, are routinely monitored.

Most feed is autoclaved prior to use. In most instances, natural ingredient diets that cannot be autoclaved are irradiated by the manufacturer. Most purified, custom diets are irradiated at the manufacturer except when requested by the investigator (compare with previous studies). The QAL checks microbial levels in all these diets.

The effectiveness of the autoclave procedure is monitored weekly using biological indicators (Verify®, Steris Corporation), and chemical indicators (Sterigage®, 3M Corporation), microbial analyses of samples by the QAL, and by maintaining a log of autoclave performance (computer printouts). In addition, autoclaved NIH-31 diet is monitored monthly for pellet hardness.

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, etc.).

A) Reverse Osmosis/ Deionized (RO/DI) Water – Animal drinking water is Durham city water that is filtered through a series of charcoal filters, a deionization resin filter followed by a reverse osmosis purification system and then stored in holding tanks. Redacted by agreement has two holding tanks (290 gallons and 505 gallons). Redacted by agreement has one holding tank (435 gallons). Water is pumped from the holding tanks directly to the bottle fill stations in the Redacted by agreement clean cage wash areas.

B) Treated RO/DI Water – Used for athymic nude mice and other immunocompromised strains (i.e. irradiated mice).

- Acidified RO/DI water used for athymic nude mice and other immunocompromised strains. Acidified water (pH 2.5-3.0) is produced in the E module clean cage wash area using an Edstrom automated water proportioner.
- Antibiotic (neomycin) treated RO/DI water used for some immunocompromised strains and irradiated mice.
- Autoclaved RO/DI water used for gnotobiotic isolators.

RO/DI and treated RO/DI water is dispensed to rodents via water bottles with sipper tubes.

C) Napa Nectar™, (Systems Engineering) or Hydrogel (Clear H_2O) is used as moisture source for transporting animals or in facility humane traps (for pest control).

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

The City of Durham provides an annual report on water quality testing (microbial and chemical) which is reviewed by the QAL. QAL tests the microbial quality of the incoming, purified and acidified water [Total plate count (TPC), coliforms and *Pseudomonas aeruginosa*] and verifies the pH of the water produced by the proportioner (acidified) weekly. Lead, copper, and iron levels are checked annually. A full contaminant screen is performed every three (3) years.

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

Automatic water delivery systems are not used for rodents.

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

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

Bedding/Nesting Material	Source(s)	Use	Animal(s)*
Sani-chips, hardwood	P.J. Murphy Forest Products, Inc.	Direct contact bedding	M, R
Diamond Soft	Envigo, Inc.	Direct contact bedding	M, R
EnviroDri	Shepherd Specialty Papers, Inc.	Nesting material	M, R,
Nestlet	Ancare, Inc.	Nesting material (not with nudes)	M
Shacks	Ketchum Manufacturing	Nesting material (veterinary/scientific purposes)	M

Rodents are housed directly on the bedding.
*M = Mice, R = Rats

ii. Describe bulk bedding storage facilities, if applicable, including vermin control measures. Note bedding storage areas within the specific animal facilities are described below in Section IV.B.4.a.

Bedding is stored with the feed in a 1284 sq. ft., air conditioned (55 to 65 ° F/50% RH) module within the Redacted by agreement warehouse is a metal building with a concrete floor. Excess pallets of bedding may be stored on metal racks in a designated area in the warehouse proper. Bulk shipments of bedding are received from the vendor every eight to ten weeks and stored on pallets.

At weekly intervals, four to six pallets of bedding are delivered to the feed/bedding holding rooms Redacted by agreement at the South Campus. The bedding is then autoclaved, cooled, and dispensed manually or via the Ducon (vacuum) system to the clean side of cage wash for distribution into cages that are transported to the animal rooms for use.

The bedding storage area has insect monitoring stations and live traps supplied with Napa Nectar that are checked daily by animal support staff.

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

Each shipment of bedding is inspected for damaged bags (tears, split seams, moisture, etc.). Approximately 10 bags of hardwood bedding per shipment are weighed to confirm contract specifications are met.

Warehouse personnel deliver three bags from each batch to the QAL for analysis of particle size (dust content) and for chemical contaminants.

Each batch of bedding is monitored for:

1. Chemical Contaminants (by vendor, report reviewed by QAL):
   a) Pesticide Screens I, II, III (same as feed).
   b) Wood Preservatives – Pentachlorophenol (PCP)

2. Physical Characteristics (by QAL): % Dust, particle size, appearance, etc.

3. Microbial content (by QAL) – total plate count (TPC)

The size of bedding particles is determined by shaking a 50-gram sample in a portable sieve shaker containing No. 8, 20, 30 and 50 U.S. standard sieves for 3 minutes. Shipments of bedding that do not pass contract specification for particle size and dust content are rejected.

Bedding is autoclaved prior to use. The effectiveness of this procedure is monitored using biological indicators (Verify®, Steris Corp.) to assure sterlization, chemical indicators (Sterigage®, 3M Corp.) to assure proper autoclave function, microbial analyses of samples by QAL, and by maintaining a log of autoclave performance (computer printouts).

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.

One cargo van with metal floor and walls is used to transport animals. A rubber floor mat is used during transport to prevent crates and caging from moving. The van is equipped with standard vehicle heating and air conditioning and has a digital temperature/humidity monitor that is checked prior to the transport of animals. No animals are transported until the van has reached the correct comfort zone for the animals. Records documenting temperature and humidity during transport of animals are maintained.

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

A Viratek Station (chlorine dioxide misting tunnel) is used to decontaminate all surfaces of incoming animal transportation crates and supplies. High pressure sprayers are used along with appropriate disinfectants for decontaminating the Ducon

feed and bedding delivery system and the animal delivery van. A SteraMist™ surface disinfection unit (TOMI Environmental Solutions, Inc.) which applies activated, ionized hydrogen peroxide is used for decontaminating animal rooms. A HEPA filtered dry vacuum is available for use in corridors. A forklift is used for moving bedding and feed pallets in the ABF Building. Fork lift operators undergo certification every three years.

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.

Rodents (solid bottom cages)

1. Static microisolator mouse cages with hardwood bedding are changed at least weekly. Static microisolator mouse cages with Diamond-Soft bedding are changed at least 2x/wk.
2. Static microisolator rat cages with hardwood bedding are changed at least 2x/wk.
3. Solid bottom cages in all mouse ventilated cage racks are changed at a maximum interval of two weeks. Solid bottom cages in all rat ventilated cage racks are changed at least weekly.
4. Cages in the gnotobiotic isolators are changed once/week.
5. Disposable enrichment (nesting material) is replaced at cage change per the Environmental Enrichment for Mice and Rats SOP.

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

Solid bottom cages in all mouse ventilated cage racks are changed using NIEHS established and ACUC approved performance standards. The maximum interval between changes is two weeks. Intracage ammonia levels were evaluated with various cage densities and strains and it was determined that the frequency of our cage change ensures that ammonia concentrations stay below levels reported in the literature to cause an animal impact (< 50 ppm).

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

All soiled bedding from untreated animals is removed from the cages in the soiled cage wash areas and emptied into a vacuum hopper (Ducon system) equipped with a hood, located adjacent to the tunnel washers. Bedding from treated animals is described below (f. waste disposal, i. soiled bedding and refuse).

Clean bedding is dispensed automatically into clean cages on the clean side of the cage wash areas.

ii. Cleaning and Disinfection of the Micro- and Macro-Environments
Describe the washing/sanitizing frequency, and methods used in the Appendix, "Cleaning and Disinfection of the Micro- and Macro-Environment."

1) Describe any IACUC/OB-approved exceptions to the Guide (or applicable regulations) recommended sanitization intervals.

There are two exceptions to the Guide recommended cage sanitation frequencies. As outlined above:

1. Solid bottom cages in all mouse ventilated cage racks are changed using NIEHS established and ACUC approved performance standards. The maximum interval between changes is two weeks. Intracage ammonia levels were evaluated with various cage densities and strains and it was determined that the frequency of our cage change ensures that ammonia level stay below levels reported in the literature to cause an animal impact (< 50 ppm)

2. Mouse cages in four semi-rigid gnotobiotic isolators receive a weekly bedding change but cages are not sanitized at weekly intervals because the CRASF or germ-free mice lack urease-positive bacteria. Monthly (germ-free) and quarterly (defined-flora) evaluations of gnotobiotic mice by QAL confirms microbial status. Identification of any bacteria in the germ-free or urease-producing bacteria in the defined-flora isolators indicates contamination. Once detected, these isolator are broken down, re-sterilized and restocked.

2) Assessing the Effectiveness of Sanitation and Mechanical Washer Function

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

Cage Washers, Bottle Washers, Rack Washers:

- A daily log is used to record water temperature using 3-Temp Thermolabel (temperature recording labels) from Paper Thermometer Co. The initial load each morning and afternoon is monitored. Monitoring is also initiated if any fluctuation in steam supply occurs.
- Equipment breakdowns, repairs, and cleaning are also noted in a maintenance log book.
- Monthly, or as needed, swabbed specimens are collected on representative samples of sanitized equipment and accessories (cages, bottles, sipper tubes, feeders) and cultured for the presence of coliforms. Any growth indicates inadequate sanitation and would be reported to ARS immediately so that corrective actions can be taken.
- QAL is currently collecting data on total plate counts (TPCs) of freshly sanitized caging materials to establish baselines and monitor overall sanitation efficiency.

b) Describe preventive maintenance programs for mechanical washers.

The Office of Research Facilities (ORF) maintains a service contract for the repair and maintenance of all animal facility washers. Preventative maintenance is provided monthly to ensure proper and efficient operation. Scheduled checks and services include but are not limited to adjustments, cleaning, calibration, inspection, and equipment servicing. All cagewash problems are repaired promptly.

f. Waste Disposal [Guide, p. 73-74]

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

i. Soiled bedding and refuse

Soiled bedding from non-treated animals: Soiled bedding is removed from the cages on the return cage wash side and emptied into the Ducon waste disposal system. Refuse is conveyed to the dumpsters and delivered to an offsite contract for composting.

Soiled bedding from animals treated with non-hazardous compounds: Soiled bedding is removed from the cages in the animal room dump station (collected in a black bag and sealed with yellow tape) and is transported daily (Monday-Friday) to the campus incineration plant where it is incinerated through a contract service. The NIEHS Campus two-chamber medical-pathological incinerator is used with a required minimum upper chamber temperature of 1800° F.

General refuse and office trash from the animal facility is bagged and placed in a dumpster by CMB contract housekeeping staff. Dumpsters are transported weekly to a landfill.

ii. Animal carcasses

Carcasses of non-treated animals and animals treated with non-hazardous compounds: Animal carcasses are double bagged, boxed, labeled, and refrigerated or frozen until disposal by on site incineration in accordance with NIH Policy Manual 3032- Waste Minimization and Management at NIH.

iii. Hazardous wastes - infectious, toxic, radioactive, sharps and glass handling, storage, method and frequency of disposal, and final disposal location

All animal carcasses, potentially infectious bedding and waste are placed in boxes, and handled according to NIH Policy Manual 3032 - Waste Minimization and Management at NIH.

Requirements for handling hazardous bedding and the appropriate waste stream are determined by the HSB and are described in the approved safety protocol and safety protocol animal addendum that accompany each animal study. General instruction

and guidance on how to safely prepare hazardous waste materials for pick-up, handling and disposal are contained in the NIEHS Waste Manual.

Biological Waste:
Cages contaminated with biohazards are double orange bagged within the room. The bags are sprayed with a QAL-approved disinfectant prior to being removed from the animal room and transported to the ABF for autoclaving. Once autoclaved, the bags are brought to Redacted by agreement opened, cages are processed through the tunnel washer, bedding is emptied into black bags, and disposed by on-site incineration.

Animal carcasses are double bagged, boxed and orange bagged, labeled, refrigerated or frozen in Redacted by agreement A HSB Waste Pickup Request for Hazardous Agents is submitted by CMB or the PI. Carcasses are handled by HSB staff as hazardous waste and are disposed by on-site incineration.

Chemical Waste:
Soiled bedding is removed from the cages in the animal room dump station, collected in a yellow bag, labeled, placed in dedicated bins in return corridors and picked up and disposed by HSB.

Animal carcasses are bagged, boxed and yellow bagged, labeled, and placed in Redacted by agreement A HSB Waste Pickup Request for Hazardous Agents is submitted by CMB or the PI. Carcasses are handled by HSB staff as hazardous waste and are disposed by on-site incineration.

Radioactive Waste:
Animal carcasses and wastes tagged with radioactive isotopes (e.g., 14C, 35S, 3H and 32P) are bagged, boxed and yellow bagged, and labeled with a "Radioactive Waste" sticker. Users may temporarily store packaged materials in appropriately marked freezers in the scientist's laboratory until collected by the HSB.

Contaminated Sharps and Glass Waste: All syringes, needles, scalpel blades, etc., are placed in approved sharps containers, and disposed as MPW. Contaminated sharps are placed intact in an impenetrable plastic container, labeled, and picked up by the HSB as MPW and incinerated on-site. Contaminated broken glass or plastic materials are packaged and sealed in a broken glass box, labeled, and picked up by the HSB as MPW and incinerated on-site.

The HSB collects hazardous waste and transports it in secure vehicles to the Redacted by agreement Redacted by agreement Waste received at the waste handling facility is separated and packaged for disposal in accordance with the NIEHS Hazardous Waste Management Permit and other applicable Federal, State and local hazardous waste regulations.

Regulated chemical waste materials are shipped to commercially permitted hazardous waste treatment/disposal facilities. Other wastes may be incinerated on site in a special high temperature medical/pathological incinerator. This incinerator, equipped with state-of-the-art emission controls, has been granted a final air quality permit by the State of North Carolina.

g. Pest Control [Guide, p. 74]

i. Describe the program for controlling pests (insects, rodents, predators, etc.) noting the control agent(s) used, where applied, and who oversees the program and applies the agent(s). Include a description of natural predators (e.g., barn cats) or guard animals (e.g., dogs, donkeys) used for pest and predator control, if applicable.

1) Program

The NIEHS pest control program involves the following areas: a) control of pests inside the animal facilities, b) control of pests inside the cafeteria, and all other buildings, and c) control of outside pests on NIEHS grounds (birds, wild rodents, etc.) Redacted by agreement North Carolina Certified Applicator in the areas of Structural Pests, Wood Destroying Pests, and Fumigation, is responsible for the pest control program within the vivarium and feed/bedding storage areas. Redacted by agreement Redacted by agreement are also North Carolina Certified Applicators and serve as back-up to Redacted by agreement Pest control for the remainder of the NIEHS campus is performed by an outside contractor. The objective of the program is to use chemicals only when necessary and in a manner that is safe for the environment, NIEHS personnel, animals, and experimental studies. The campus pest contractor informs Redacted by agreement when any chemical treatments are performed or wild rodents are captured.

a) Control of Pests in the Animal Facility

1. Animals Rooms

Each animal room contains one live trap (supplied with Napa Nectar) and one insect monitoring station. Additionally, Illinois cubicle rooms contain one insect monitoring station per cubicle. Animal technicians check traps daily and findings are logged on the "Pest Control Form" in each animal room logbook. Positive findings of rodents, roaches, or excessive numbers of small insects are reported immediately to the Facility Manager (ARS). The "Pest Control Form" for each animal room is submitted to the Facility Manager monthly. The Facility Manager reviews the reports, responds as needed, and completes the Monthly Pest Control Monitoring Report which is maintained on the CMB server. Abnormal results are presented to the CMB staff at biweekly CMB Operations meetings.

2. Facility Support Areas

Live traps (supplied with Napa Nectar) and insect monitoring stations are present in procedure/necropsy areas, storage areas, washrooms, corridors, and receiving areas throughout the animal facility and are monitored by the animal husbandry support contract staff. Insect monitoring stations are present in office areas. Live traps are checked daily while insect monitoring stations are checked Monday-Friday. Positive findings of rodents, roaches, or excessive numbers of small insects are reported immediately to the Facility Manager. All findings are logged on the "Pest Control

Monitoring Report". Each completed "Pest Control Monitoring Report" is submitted to and filed by the Contract Project Manager.

3. Animal Feed and Bedding Storage Areas (Animal Bedding and Feed (ABF) Room Redacted by agreement Redacted by agreement warehouse and all enclosed feed and bedding holding areas in Redacted by agreement Redacted by agreement are monitored daily by the animal husbandry support contract staff. Live traps are supplied with Napa Nectar, which is replaced by the Animal Husbandry Support Contract Staff when expired (expiration date on pouch) or is opened. Insect monitoring stations are used to monitor for cockroaches, ants, and silverfish, etc., and are replaced monthly or more often as needed. Both clean and return areas in the ABF have insect lights. Positive findings of rodents, roaches, or excessive numbers of small insects are reported immediately to the QAL. All findings are logged on the "Pest Control Monitoring Report".

Proper sanitization and construction/facility design are the primary pest control measures. An integrated pest management approach is used to limit pesticide and chemical bait use, which are not routinely used in the animal facility. However, if pests are identified the following products may be used:

- Maxforce© Ant killer bait (Hydramethylnon)
- Optigard Ant Gel Bait (.10% Thiamethoxam)
- Pyrethrum - Used as a flushing agent in wash rooms and storage areas to pinpoint pest problems.
- Boric acid powder or Perma-Dust-Used as a preventative in occult spaces during construction and remodeling. Used as a treatment in selected areas under sinks, door, cabinets, etc.
- Carbamates (Baygon, Invader, Sevin) -Applied in selected areas outside the animal holding rooms (storage areas, bathrooms, washrooms, corridors, etc.) if needed for quick effects.
- Pyrethrum/Synthetic Pyrethrum (Demon) - Used for crack and crevice and selected spot treatments in wash rooms, storage areas and corridors.

The date of application, chemical, concentration, method of application, target pests, and area treated are all recorded and filed in the QAL.

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

Animals are not exposed to pesticides. If pests became an issue in an animal room, the facility manager would review pest control options with the principal investigator to develop a course of action.

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

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

and what procedures are performed. Indicate qualifications of weekend/holiday staff if not regular staff.

Animal care personnel provide weekend and holiday coverage. A minimum of 5 contract animal technicians, including one supervisor, and one breeding technician are on duty each weekend and holiday for approximately four hours/day. One government supervisor and one veterinarian are on call on weekends and holidays and are available by phone. A weekend instruction sheet detailing studies and colonies that require special monitoring is prepared by CMB during the week and provided to all weekend staff (animal technicians, supervisors, veterinarians). The weekend instruction sheet includes CMB personnel phone numbers, emergency contacts, etc. Animal health, caging, water, food, and environmental conditions are checked daily. Dosing and treatments required to support special studies are performed as needed by government technicians and contract personnel.

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

Procedures for contacting responsible veterinary personnel are outlined in the weekend instruction sheet and posted in the animal facility. Emergency call lists are also posted in the engineer's office and in the security office. On-call personnel may be contacted by phone 24 hours a day.

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, etc.).

Rodents may be individually identified by cage card, ear punch, tattoo, or with an implantable microchip. Cage cards prepared by the CMB office are affixed to each cage. Cage cards contain the following information: bar code, requisition number, room number, PI, branch, using investigator, ASP number, species, vendor, vendor location, strain, sex, age/weight, date of birth, and number/cage. Research notations such as type and date of surgery, compound dosed, date mated, etc. may be added by the investigator.

b. Record Keeping

Describe procedure(s) for maintaining individual records on animals. Identify the species for which individual records are maintained, individuals (titles, not necessarily names) responsible for maintaining the records, and where they are maintained and how veterinary and IACUC/OB access is assured.

VMS maintains records of individual animals referred for veterinary monitoring. Completed health records are kept in the VMS Laboratory [Redacted by agreement] A blue color-coded card indicating the treatment regimen is attached to the individual cage during any period of treatment or monitoring. Veterinarians and ACUC members have access to these records.

Breeding records for colonies maintained by CMB are available on the colony management server. Records are accessed and maintained by the CMB breeding technicians and laboratory staff.

Other records, such as documentation of experimental procedures and surgeries are the responsibility of the investigator. ACUC-required monitoring logs, including surgical, weight, post-operative logs are maintained at the room level during post-procedural monitoring and in the laboratory. The VMS has access to the logs and the ACUC reviews logs during the semi-annual laboratory site visits.

c. Breeding, Genetics and Nomenclature

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

Investigators are provided genetic histories from the animal vendors or collaborating investigators. Consultation with veterinary staff, ACUC office staff, and a molecular geneticist in the NIEHS Knock-Out Core is provided. A CMB contract breeding specialist is available for consultation.

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.

CMB houses all animals for investigators. Appropriate nomenclature on all cage cards is completed before an investigator receives the animals. Applicable guidelines such as those from the International Committee on Standardized Genetic Nomenclature for Mice and the ILAR documents, "Standardized Nomenclature for Transgenic Animals" and "Definition, Nomenclature, and Conservation of Rat Strains" are used to advise investigators of proper nomenclature.

The NIEHS ACUC has a guideline to assist investigators in utilizing proper nomenclature for genetically altered mouse strains. Proper nomenclature is requested in the ASP/amendments. Shorthand nomenclature may be used on cage cards and is also available in the ASP/amendment. CMB maintains a database which tracks proper and shorthand nomenclature. ACUC office staff and a molecular geneticist in the NIEHS Knock-Out Core are available to assist investigators research and formulate the proper nomenclature and appropriate shorthand names that can be used for cage cards.

iii. For newly generated genotypes, describe how new phenotypes that negatively impact well-being will be monitored, managed and reported to the IACUC/OB in a manner to ensure the animals' health and well-being.

Based on knowledge of the gene(s) of interest or related genes, expected phenotypes of genetically modified animals (GMA) are fully described in the ASP/amendment. The PI and CMB veterinarian develop a customized monitoring plan to ensure that the development of an adverse phenotype does not impact animal welfare. The PI outlines the monitoring frequency, procedures, and humane endpoints in the ASP.

When GMAs are newly generated with unpredictable phenotypes, the VMS monitors these animals and reviews PI generated documents (i.e. body weight logs) as requested by the ACUC. Animal husbandry and breeding technicians monitor mice daily and report any clinical signs to the VMS. The breeding specialist prepares a Breeding Summary Sheet for each colony which outlines adverse phenotypes. VMS documents the development of an unanticipated clinical condition. In consultation with a CMB veterinarian, treatment or intervention strategies are discussed and humane endpoints are developed relating to this unanticipated clinical condition. VMS summarizes clinical observations via a MTR to the ACUC office. If a new phenotype results in a change to the pain or distress category, an amendment is required that describes the new phenotype and clinical condition, treatment/intervention strategies, humane endpoints and monitoring refinements, reclassification of the pain and distress category and alternative justification submission.

Various methods are used to refine endpoints and manage adverse phenotypes. When possible, PIs use recombination systems such as Cre-LoxP and FLP-FRT to limit gene expression to certain tissues or inducible promoters to control expression in time dependent fashion. Other management techniques include termination of the study before the age when the phenotype causes a welfare problem (i.e. early retirement of breeders/adults, early genotyping and euthanasia of pups), special husbandry procedures such as mash diet/soft bedding in support of a neurological phenotype, treatment of dermatitis, cross fostering in the case of maternal phenotypes, and altered breeding schemes to minimize production of mice with the adverse phenotype (i.e. changing the background strain or breeding phenotypically normal heterozygotes).

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

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

A. Animal Procurement and Transportation [Guide, pp. 106-109; Ag Guide, pp. 8; 45; 51-57]

1. Animal Procurement

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

Animals imported into the NIEHS Redacted by agreement primary vivarium are required to be free of all murine pathogens listed on the NIEHS Animal Pathogen Bioexclusion List (Appendix 26). Sources are evaluated and selected based primarily on their historical data, use and quality control record. Vendor health surveillance data is reviewed and supplemented by in house evaluations and consultation with other users. Periodic site visits of vendor facilities are conducted by CMB professional staff or by NIH officials who furnish the QAL with their reports. The CMB staff communicate with vendor representatives on a regular basis.

When considering a new animal source, a history of the supplier is researched, discussions are held with the supplier's health professionals. Once the health history is approved, initial orders

from a new source may be housed in isolation from the resident population, and their health status verified. Only after the source is certified as pathogen-free would animals be ordered for housing. Only well-established suppliers are routinely used.

Frequently, rodents, particularly genetically engineered mice, cannot be supplied from approved commercial vendors and are requested from other institutions. Discussions with the outside institute's health professionals include obtaining information on colony size, location, husbandry techniques, health surveillance data and disease history. The current policy is to rederive all animals from non-standard sources. Contracts have been established with major animal vendors for rodent rederivation or maintenance. Receipt of the offspring from rederivation/embryo transfer procedures is dependent upon review and approval of health surveillance data.

One contract is in place for the development of transgenic and conditional knock-out mice (Xenogen Biosciences, Inc./Taconic). This requires ongoing comprehensive, in-depth monitoring of health status and CMB approval prior to receipt of each shipment.

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).

The NIEHS animal care program follows the standards set in the ARAC Guidelines for NIH Rodent Transportation with further refinements detailed in the NIEHS guidelines for Shipping Animals from NIEHS to Other Institutions, Transportation of Animals to Redacted by agreement Laboratories and Local Institiutes by CMB, and Transportation of Animals to Redacted by agreement Laboratories by Investigators/Lab Technicians.

All rodents are transported to NIEHS from approved vendors in filtered animal crates by vendor transportation using the most appropriate modes of transportation (flight, climate-controlled truck). The condition of the animal transportation van and the animal crates are observed at the time of delivery.

After unloading, crates are placed through a misting tunnel (delivering chlorine dioxide) which decontaminates all the outer surfaces of the containers. The misting tunnel is in a temperature controlled receiving area adjacent to the loading dock. After being sprayed, the crates are delivered to the animal facility by freight elevator located in this receiving area. Animals are uncrated and evaluated (health, order specifications, etc) in the animal room by the animal technician and placed into home cages.

Rodents transported from the NIEHS animal facility to laboratories in the same building are transported in filter-topped microisolator cages or IVC by CMB personnel or approved laboratory personnel.

All local, off-site transportation of rodents is performed by CMB using appropriate containment (polycarbonate micro-isolator cages or filtered crates) and a climate-controlled vehicle. Long distance transportation arrangements are made by CMB using approved couriers. Rodents are shipped in filtered crates with appropriate rodent chow/gel packs, nesting material for enrichment, and bedding. All animals transported locally or long distances are evaluated by a CMB veterinarian and a health report is completed before shipment.

B. Preventive Medicine

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

a. Describe methods used to monitor for known or unknown infectious agents.

In the NIEHS Redacted by agreement primary vivarium, sentinel rodents are used as the primary method for ongoing health surveillance of the research rodent population. Gnotobiotic (Charles River Altered Schaedler Flora -CRASF), CD-1, or Swiss Webster mice are used as sentinels and are produced in-house in semi-rigid isolators. These isolators are screened on a quarterly basis by the QAL. Fecal specimens and swabs of internal isolator surfaces are evaluated for the presence of aerobic bacteria. The CRASF populations are replaced if contaminated with urease-positive bacteria (e.g., Proteus spp., Klebsiella spp.)

Sprague Dawley outbred [Crl:CD(SD)] or inbred F344/DuCrl rats are used as sentinels in Redacted by agreement rat rooms and are purchased from an approved vendor as needed.

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

Stringent review of all animal procurement requests prevents entry of infectious agents to assure the animals are free of all organisms on the NIEHS Animal Pathogen Bioexclusion List (Appendix 26). Additional safeguards include the rederivation of lines originating from non-approved sources, testing of all cell lines, blood products, and other biologics destined for administration into animals, and procedures prohibiting animal return to the animal facility. Maintenance of a centralized facility with clearly defined clean and soiled areas, appropriate flow of personnel and supplies, a comprehensive pest control program, and extensive practices and procedures to ensure appropriate sanitation of supplies, equipment, and facility minimizes the entry of infectious agents.

Detection of infectious agents rest with a rigorous sentinel surveillance program and daily animal observations by trained animal technicians with veterinary oversight. If a pathogen is detected, quarantine procedures are immediately instituted as described in the animal pathogen detection SOP. The use of dedicated PPE and static microisolator or ventilated caging systems aids in agent containment. The method for elimination of the infectious agent depends upon the agent, population of animals infected, and purpose of the study and may include rederivation or culling.

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

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

Accompanying animal shipment paperwork is evaluated for accuracy (i.e. strain, sex, number, and source). Upon arrival in the animal facility each crate is opened in the animal room hood and animal technicians examine the animals to evaluate their health status. Appropriate strain, sex, number, and source are rechecked.

b. Describe quarantine procedures for each species that are purpose bred.

All incoming animals are purpose bred. Selected supplier stock is allowed into the facility from CMB approved sources and no additional quarantine is routinely performed. All mice generated by in-house embryo transfer from outside sources are quarantined in ventilated cage racks housed in an Illinois cubicle room until health status is cleared through the QAL and the mice can be moved to the general population.

c. Describe the quarantine facilities. In your description explain any special measures used for quarantine/conditioning of each random source (not bred and raised specifically for research) species used.

An Illinois cubicle is available to house animals requiring health status confirmation. No random source animals are used.

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

Investigators are encouraged to allow rodents to acclimate for a minimum of 48 hours. Most are acclimated for 7-10 days.

e. 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.

Animal species are separated at room level.

All incoming animals are purpose bred. Rodents allowed into the facility are from CMB approved sources and are of similar health status, which are free of all pathogenic agents listed on the NIEHS Rodent Pathogen Bioexclusion List- Redacted by Vivarium agreement (Appendix 26).

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

a. Describe isolation procedures and related facilities for animals.

Animal species are separated at room level. If a disease outbreak were to occur animals would be quarantined at the room level. Suspect animals may be further quarantined in an Illinois cubicle.

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

Not Applicable. Animal species are separated at room level.

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

a. Describe 1) the procedure(s) for daily observation of animals for illness or abnormal behavior, 2) the observer's training for this responsibility, and 3) method for reporting observations (written or verbal). Include a description of the method for ensuring that reported cases are appropriately managed in a timely manner.

Animal care personnel observe each animal daily. Animal health checks are documented in a logbook outside of each animal room.

Animal care personnel are trained through individualized training and group training sessions in recognizing signs of disease and the normal and abnormal behavior of the animals at NIEHS. In addition, all animal care staff are required to obtain AALAS certification.

Any abnormalities, illness, reproductive problems, or deaths are reported to the VMS in writing, by submission of an Animal Health Report Form. There is an SOP for reporting animal health concerns. VMS evaluates the Animal Health Report Forms throughout the business day and cases are triaged and examined in a timely manner. If the animal(s) require immediate attention, the VMS or first available CMB veterinarian is contacted verbally by phone or in person.

b. Describe the methods of communication between the animal care staff/veterinarians and the researcher(s).

A CMB Animal Disposition Form, submitted by the lab and maintained at the room level, provides technical and veterinary staff contact information for investigators and instructions regarding animal disposition.

The animal technician reports all health problems by placing a blue card in the cage cardholder and submitting a completed Animal Health Report Form to the VMS. A veterinary technician responds by evaluating the animal. If moribund, the animal is euthanized; otherwise the investigator is contacted via email, telephone, or in person depending upon the severity of the case to determine if treatment is an option. Veterinary consultation occurs for unique presentations and when standardized interventions are not effective. VMS conveys the treatment plan in writing to the animal technician and treatments are documented on the blue card at the cage level. The researcher is kept apprised on the animal's condition when treatment or monitoring is done.

On weekends, the animal technician speaks directly with the on-call veterinarian regarding any animal or study concerns.

c. Describe the procedure for providing veterinary medical care to ill animals and note who is contacted and the method of communicating (written or verbal) information to the veterinarian regarding sick animals.

VMS provides intervention on routine clinical cases. The VMS technician determines whether to monitor, treat, or euthanize the animal based upon information provided by the investigator and a veterinarian. Veterinary consultation occurs for unique presentations and when standardized interventions are not effective. If further observation is warranted, the blue card, as described in b. above, is left on the cage and instructions for monitoring (i.e., parameters monitored, monitoring frequency) are added by the veterinarian or the VMS technician. If treatment is required, treatment instructions (i.e., therapy to be administered, route, frequency) are indicated by the VMS tech/vet on the blue card. The animal technician performing the treatment records the treatment given on the card, initials, and dates. The veterinarian or VMS technician notes the progress and plan on the Animal Health Report Form. At case resolution, the completed Animal Health Report

Form is submitted to the module supervisor and the PI with the veterinarian retaining the original in the VMS lab. The blue card remains on the cage (placed behind cage card) after the case is resolved for future reference, if needed.

d. 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, etc.) for each species.

The preventive medicine program is based primarily upon control of vendor source. Animals may only be brought into NIEHS facilities from approved vendors or by special approval from CMB. Several methods are used to monitor approved vendors including supplier health status reports, communication with other user groups who monitor vendors such as NCI, periodic in house monitoring of incoming animals, pre-screening of any animals from a new vendor, site visit by CMB or NIH personnel to vendor's facilities, and in house sentinel animal surveillance program. In addition, testing of all biological materials (i.e. cells, tissues) for the presence of murine pathogens (via culture or PCR) is required prior to animal inoculation. VMS examines all potential disease problems.

A sentinel monitoring program managed by the CMB QAL is in place.
As described in section B.1.a, gnotobiotic (defined-flora) mice are raised on-site for use as sentinels. Two (2) sentinel mice are placed on every rack used. Dirty bedding from all cages on the rack is transferred to the sentinel cage at each cage change. Testing for murine pathogens is performed on sentinel mice monthly with survival blood collection performed on months 1 and 2 of the quarter for serological analysis of high prevalence viruses (see Appendix NIEHS Serological Panels). A full necropsy is performed on one (1) sentinel mouse/rack at the end of the quarters (months 3 and 6). Sentinel pairs are replaced every six (6) months. Every quarter the following tests are performed:

1. A visual pelt exam for ectoparasites.
2. Cecal/colonic contents are examined for endoparasites
3. Fecal PCR for Helicobacter spp.
4. Oropharyngeal culture
5. Fecal culture
6. Every six (6) months, a fecal PCR is performed for murine pinworms.

Crl:CD(SD) or F344/DuCrl rats are used as sentinels in rat rooms and are purchased from an approved vendor. They are processed and tested for the presence of infectious agents on a quarterly basis. Serological panels performed are listed in the NIEHS Animal Health Surveillance Program SOP. In addition, the following tests are performed quarterly:

1. A visual pelt exam for ectoparasites.
2. Cecal/colonic contents are examined for endoparasites
3. Fecal PCR for Helicobacter spp.
4. Oropharyngeal culture
5. Fecal culture
6. Every six (6) months, a fecal PCR is performed for murine pinworms.

Environmental testing for infectious agents such as PCR analysis of swabs taken from IVC cages or racks is currently being performed on a case-by-case basis. Samples for

culture or PCR are collected from any clinical case in which an infectious agent is suspected.

C. Clinical Care and Management [Guide, pp. 113-115]

1. Emergency Care [Guide, p. 114]

a. Describe the procedures to ensure that emergency care is continuously available for animals during and outside of regular work hours.

Veterinary care is available 24/7. Animal care staff including animal technicians, veterinary technicians, and veterinarians are available in person, by phone, or email during regular work hours. Veterinarians rotate on-call weekends and holidays. Redacted by agreement

Redacted by agreement

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

The NIH Policy Memo: Timely Assessment and Resolution of Animal Issues Involving Potential Pain and Distress expresses the DDIR/IO's expectations for daily monitoring, communication, response and care of the research animals to include the attending veterinarian's responsibility and authority.

NIEHS has expanded upon the policy memo "Timely Assessment and Resolution of Animal Issues Involving Potential Pain and Distress" by initiating the use of an experimental summary sheet and CMB Animal Disposition Form. Both documents are submitted with each approved ASP and amendment. All veterinary technicians and veterinarians have the authority to provide emergency treatment to research animals as required. Investigators provide a CMB Animal Disposition Form and an Experimental Studies Summary Sheet, which indicates special instructions for handling animals. Every attempt is made to contact investigators and to work with investigators to achieve scientific requirements along with animal welfare, but any veterinarian or veterinary technician has the authority to appropriately treat (including euthanasia) any animal in a timely manner to relieve potential pain or distress.

2. Clinical Record keeping [Guide, p. 115]

Describe the procedure for maintaining medical records and documenting treatment of ill animals including: clinical laboratory findings, diagnoses, treatments, medical progress records, etc. Identify individual(s) (titles, not necessarily names) responsible for maintaining such records and identify where the records are maintained and who has access to the records. Describe the role of the Attending Veterinarian in record keeping.

A blue cage card marks cages identified for monitoring or those requiring treatment. The blue card is left on the cage and instructions for monitoring or treatment (i.e., parameters monitored, monitoring frequency, therapy to be administered, route, frequency) are added by the veterinarian or the VMS technician. The animal technician performing the treatment records

the treatment, initials, and dates the card. The blue card remains on the cage (placed behind cage card) after the case is resolved for future reference, if needed.

An animal health report form is concurrently completed by the animal technician and submitted to VMS. The veterinarian or VMS technician notes the progress and plan on the Animal Health Report Form. At case resolution, the completed Animal Health Report Form is submitted to the module supervisor and the PI with the veterinarian retaining the original in the VMS lab. An Animal Health Report Form accompanies sick or dead animals sent to the VMS Laboratory. The Animal Health Report Form summarizing all findings including clinical pathology, microbiology, parasitology, pathology, and final diagnosis is completed by the veterinary technician and approved by the clinical veterinarian.

Original reports are retained in the VMS Laboratory. Upon resolution, the investigator is sent a copy of the form. Summaries of all animal health reports submitted to the VMS are centered into a database system which can be sorted by various means (room, strain, investigator, condition etc.).

The AV establishes clinical record keeping policies and procedures. The VMS clinical veterinarian assumes overall responsibility for medical records. The veterinarians (including the Attending Veterinarian), VMS technicians, supervisors and the facility manager have direct access to the records.

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

a. In-house diagnostic laboratory capabilities.

In addition to a complete visual and physical examination, a full range of routine diagnostic capabilities is available. The VMS Laboratory is equipped with a Heska chemistry analyzer which can run a small panel of serum chemistries (BUN, creatinine, glucose, serum ALT, SAP, and total protein). Complete urinalysis, blood smear examination, and fecal examination can be done.

QAL is a functional microbiology testing laboratory and performs in-house bacterial and fungal culturing and identification including matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) spectrometry, Polymerase Chain Reaction (PCR) assays and genomic sequencing.

Specialized clinical pathology and complete blood analyses are available in-house from the NIEHS Cellular and Molecular Pathology Branch.

b. Commercially provided diagnostic laboratory services.

Commercial laboratory assistance (via contracts) is used for viral, mycoplasma and bacterial screening of rodent specimens, for nutritional analysis and chemical contaminant (heavy metals and pesticides) screening of animal feeds, and for screening of chemical contaminants in bedding. Specialized clinical pathology procedures are also available from a commercial laboratory (i.e., clinical chemistries) as well as from the NIEHS Cellular and Molecular Pathology Branch.

c. Necropsy facilities and histopathology capabilities.

Complete necropsy facilities are available in in the animal facility Redacted by agreement Redacted by agreement as well as in the VMS Laboratory Redacted by agreement and in the QAL Redacted by agreement Redacted by agreement Full gross and microscopic evaluations, including EM if necessary, are available through the NIEHS Cellular and Molecular Pathology Branch (CMPB) by board certified veterinary pathologists. A pathologist is assigned to CMB which streamlines communication and processing of clinical cases submitted for histopathology.

d. Radiology and other imaging capabilities.

CMB has a Piximus bone densitometer, and a body mass analyzer (Bruker Minispec) to provide imaging services to the Institute. A Vevo 3100 High Resolution Ultrasound Biomicroscope is also available to provide additional means of non-invasive rodent imaging. An In Vivo Imaging System (IVIS) offers bioluminescence and fluorescence imaging capabilities.

4. Drug Storage and Control

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

Controlled and non-controlled substances are purchased from commercial vendors or through an accredited compounding pharmacy. Controlled substances (Schedules II-V) are purchased centrally by the Comparative Medicine Branch, using the NIEHS research DEA license. Controlled drugs are stored in a safe and are the responsibility of the Head, VMS, who serves as the NIEHS Controlled Substance Officer (CSO). Investigators approved to receive and use controlled substances are trained on proper record keeping and security of the drugs, requiring storage in a locked location. Once dispensed, controlled substances are stored using a double lock system. The NIEHS Guideline for the use of controlled substances is distributed to those receiving and using controlled substances. Non-controlled drugs are stored in the surgical suites (non-refrigerated storage) and the VMS laboratory (refrigerated storage).

b. Describe record keeping procedures for controlled substances.

The NIEHS CSO has overall responsibility for record keeping associated with controlled drugs. Each investigator approved to receive and dispense controlled substances for his/her laboratory is required to keep records and submit an annual inventory to the CSO. The ACUC reviews laboratory records of controlled substance use during the semiannual review of laboratories. The CSO also performs an annual inventory of the Institute's stock controlled substances.

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

1. Pre-Surgical Planning [Guide, p. 124]

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 postoperative care.

The pre-surgical planning phase begins with a veterinary consultation during ASP preparation. The ASP describes surgical procedures, including selection of anesthetic, analgesic agents, peri/postsurgical monitoring and care, and identifies the personnel who will perform these duties. During the ASP review process, the ACUC reviews the qualifications of personnel wishing to perform either survival or terminal surgical procedures.

The surgical technique of new technicians and investigators wishing to perform surgery is directly observed and evaluated by one of the veterinarians or technicians in the VMS. An MTR is filed to document proficiency and is maintained by the ACUC office.

VMS oversees the surgical areas in the animal facility and is available to assist investigators with locating equipment, supplies, and instructing them in proper use of the surgical facilities. Supplies for all surgical procedures are available from VMS.

2. Surgical Facilities [Guide, p. 116]

a. List building name(s) and room number(s) or other locations (coded, if confidential) where surgical procedures are performed. Include areas where surgical procedures are conducted in agricultural species. Indicate the type of species, nature of procedure (major/minor/emergency; survival and non-survival, etc.). Indicate for each surgical area if the use is heavy (daily), moderate (weekly), or light.

Surgical Room Redacted by agreement is used primarily for survival surgical procedures and a few non-survival surgical procedures in rats and mice with moderate usage for major and minor survival surgeries.

Surgical rooms Redacted by agreement are used for survival stereotaxic surgical procedures in mice and rats with heavy usage.

Room Redacted by agreement is used for stereotaxic surgical procedures and neurobehavior experiments in mice with moderate usage.

Room Redacted by agreement is used for survival and non-survival surgery on mice and rats with moderate usage.

Procedure Rooms Redacted by agreement are used for minor procedures in rodents (tail biopsy, injections, blood collection) with heavy usage but can be scheduled and used for nonsurvival surgery.

Room Redacted by agreement is used for embryo transfer and the non- invasive Non-Surgical Embryo Transfer – NSET procedure with moderate usage.

b. List the major surgical support equipment available at each location where survival or nonsurvival surgery is performed (e.g., gas anesthesia machines, respirators, etc.).

Each dedicated surgery location has at least one gas anesthesia machine and a supplemental heat source. Redacted by agreement has a surgical microscope and heating blankets. Redacted by agreement has a small warming pad with gas anesthesia available in Redacted by agreement Two rodent ventilators

are available for use. A physioSuite system is available in Redacted by agreement which combines a ventilator and anesthetic delivery system with anesthetic monitoring (pulse/ox).

c. Describe any specialized considerations for designation of surgical areas (e.g., rodents, aquatics, farm animals, etc.).

Redacted by agreement are dedicated surgery rooms. Procedure rooms can be used for survival rodent surgery and are scheduled and dedicated to surgery during that time.

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, etc.).

A major surgical procedure is defined in the Guide as one that "penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection." This would include laparotomy, thoracotomy etc. NIEHS considers cannulations into the brain in rodents to be a major surgical procedure.

Minor survival surgery "does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy…". Subcutaneous pellet implantation is a common minor surgical procedure performed at NIEHS. Less commonly performed procedures classified as minor surgery include subcutaneous tumor implantation, subcutaneous ECG radio telemetry implantation and subcutaneous minipump implantation.

The ACUC considers the species, health status, and age of the animal, as well as the potential for pain and postoperative complications, the nature of the procedure, the size and location of the incision, and the duration of the procedure when categorizing any surgical procedure.

b. How is non-survival surgery defined?

A non-survival procedure is one in which the animal is euthanized before recovery from anesthesia. Sterile technique is not required for non-survival procedures. At a minimum, the surgical site is prepared with alcohol, the surgeon wears gloves, and the instruments and surrounding area are clean.

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

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

Preparation of the patient includes hair removal and surgical site skin disinfection using an iodine/chlorhexidine compound followed by alcohol. Surgeon preparation includes hand washing and aseptically donning sterile gloves in addition to gown, head cover, mask, and

booties. Instruments and implantable materials such as catheters and pumps are sterilized. Surgical techniques are such that the likelihood of infection is minimized.

b. Describe methods used to sterilize instruments and protective clothing. Indicate how effectiveness of sterilization is monitored and, if applicable, any approved alternate methods for instrument re-sterilization between serial surgeries. If used, include a description of approved liquid sterilants and instrument exposure time(s) required for each.

Instruments are sterilized by steam/heat sterilization in Steri-Paks which contain a color indicator. Packs are used for surgery only if the appropriate color change indicating sterilization has occurred. Instruments used for a series of similar surgeries on a single group of animals are disinfected between animals using a dilute chlorine dioxide solution with appropriate contact time (10 minutes) and rinsing in sterile saline or by using a glass bead sterilizer. When a glass bead sterilizer is used, care is taken to ensure that instrument surfaces have cooled sufficiently before contacting animal tissues unless it is being used for cautery (such as ovariectomies). The surgeon is required to wear a disposable laboratory coat or gown, head cover, shoe covers, mask, and sterile surgical gloves. Autoclaves used for surgical pack sterilization are checked monthly with a biological indicator.

An Ethylene Oxide gas sterilizer unit for sterilization of delicate instruments is available at the U.S. Environmental Protection Agency campus located within 0.5 miles of NIEHS.

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

The VMS provides surgical support to all animal users. Veterinarians and surgical technicians are available for surgical consultation. VMS trains, monitors, and documents proficiency of those individuals on ASPs that perform surgical procedures.

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.

The anesthesia, surgery, and post surgery recordkeeping SOP establishes requirements (including documentation) of anesthetic/surgical monitoring). Group records are maintained for short procedures (less than 10 minutes) and individual logs maintained for animals undergoing longer procedures. Palpebral and pedal reflexes are used to monitor anesthetic depth as well as respiratory pattern for both survival and non-survival surgeries. Supplemental heat is provided to maintain body temperature during lengthy procedures. Subcutaneous fluids are administered during lengthy procedures or when procedures are likely to reduce post-operative fluid intake. An SOP has been developed for the Flexivent, which utilizes a neuromuscular blocking agent and where ECG or blood pressure monitoring is not possible.

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.

Responsibilities for post-operative care are shared between the veterinarians, VMS technicians, investigators, and animal care staff. Post-operative care varies with the surgical procedure. Animals must be continuously monitored until fully recovered from anesthesia. Most animals undergoing surgery are recovered on supplemental heat. Animals are monitored at least once daily for thermoregulation, post-operative pain and distress. The provision of analgesia, supportive care, wound care, etc. is detailed in the ASP. VMS maintains surgical and postoperative records electronically. For those procedures where the investigator performs the surgery, the individual laboratory maintains records.

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

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

During the ASP review process, the ACUC and veterinary staff carefully evaluate the pain and distress categorization based upon the PI's description and the ACUC's knowledge of the procedures involved. NIEHS applies USDA pain and distress categories to all animals, including rodents. Daily monitoring of the animals with close veterinary oversight ensures that the ACUC's assessment and categorization is appropriate.

2. Describe how the IACUC/OB ensures that unnecessary pain and distress are avoided (e.g., pilot studies, monitoring by veterinary staff, animal use protocols, humane endpoints, other refinements, etc.).

The ARAC "Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation", and "Guidelines for Endpoints in Animal Study Proposals" are resources for use by veterinary staff members, investigative staff, and ACUC members.

The ASP must indicate any potential adverse effects expected and include humane as well as scientific endpoints. The investigator is required to consider alternatives and refinements such as administration of analgesia or pilot studies and dose range finding studies. VMS monitors pilot studies and new studies. Technical proficiency of the investigator is monitored and documented. VMS reports observations to the ACUC office via a MTR and monthly summaries are made at the convened meeting of the ACUC.

The ACUC has approved SOP's or guidelines which have been developed to minimize pain and distress. The NIEHS ACUC also uses the guidelines developed by the NIH ARAC.

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

1. List the agents used for each species. Dosages, routes of administration and drug combination should be included in guidelines and available at the time of the site visit. Describe also any non-pharmacologic means used to diminish pain and distress.

Injectable Systemic Anesthetic Agents:
Ketamine xylazine- rodents
Sodium pentobarbital® – rodents (acute terminal procedures i.e. perfusion, exsanguination)
Urethane – rodents (terminal Flexivent procedure only)

Local Anesthetic Agents:
Topical lidocaine/prilocaine - rodents
Injectable bupivacaine-rodents

Inhalant Anesthetic Agents:
Isoflurane and oxygen - rodents
CO_2 - rodents (terminal procedure only)

Analgesics:
Buprenorphine – rodents
Buprenorphine SR- rodents
Carprofen- rats
Flunixine – rodents
Meloxicam - rodents

Non Pharmacologic Means to Minimize Pain and Distress:
Subcutaneous fluids
Mash (ground feed mixed with RO/DI water) provided in a petri dish
Supplemental heat
Soft bedding (i.e. Diamond Soft)
Additional environmental enrichment (additional nesting material).

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

All animal users complete the mandatory training "The Humane Care and Use of Animals in Research" upon initiating animal work and complete the online refresher training triennially. These courses discuss pain, distress, monitoring, and relief using anesthetics, analgesics, and other supportive care measures.

Information on drugs and techniques used to reduce pain and distress is available to investigators and technicians on the CMB webpages. The VMS has established SOPs for most commonly performed procedures which include recommended anesthetics and analgesics. These SOPs also list post-procedural care with clinical signs that may be indicators of pain/distress.

Consultation between veterinarians and research staff regarding the use of anesthetics and analgesics occurs during the planning and implementation of research experiments. Veterinarians review all ASPs including the choice of anesthetic and analgesic and the dosage and route. The importance of preemptive analgesia is discussed with all personnel performing surgical procedures. Guidance is given as to supportive care that may be beneficial such as supplemental heat/fluids or mash.

3. Describe the monitoring of the effectiveness of anesthetics and analgesics, including who does the monitoring.

The use of anesthetics and analgesics including trained personnel responsible for monitoring the effectiveness of these drugs is approved in the ASP. All investigators are required to monitor and document reflexes (palpebral and pedal) at regular intervals during procedures

where animals are anesthetized and postoperatively to ensure adequate recovery and pain management. VMS staff are available for consultation, as needed.

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.

Veterinarians and the ACUC carefully review studies requiring use of neuromuscular blocking agents. Neuromuscular blocking agents are only used for terminal procedures (FlexiVent) under deep anesthesia. Prior to administering the neuromuscular blocking agent, the depth of anesthesia is assessed by lack of pedal and palpebral reflex. VMS has ensured that deep anesthesia is maintained throughout the length of the procedure by performing the procedure without using the neuromuscular blocking agent. Results are documented in performance data collected on 3/25/16.

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

All anesthetic delivery systems are non-rebreathing. Vaporizers are serviced every three years. Vaporizers were last serviced in May 2017. An SOP for the Use of the Isoflurane Anesthesia System for Rodents has been established and is available to all investigators using this system. All volatile anesthetics must be appropriately scavenged which may include the use of a fume hood (available in procedural areas, containment rooms, Illinois cubicles) or scavenging device (i.e., f/air canister). The HSB monitors the effectiveness of scavenging procedures being utilized at least annually or more frequently, if requested.

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

1. Describe approved methods of euthanasia, including humane slaughter. Include consideration of species, age, condition (e.g., gestational period, or neonatal) and location(s) for the conduct of the procedure.

Methods of euthanasia are approved in each ASP and must be consistent with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition unless a scientific justification has been approved. Approved methods of euthanasia consider species, age, condition, and location where the procedure will be performed and are summarized below. Euthanasia may be performed in the NIEHS animal facility procedure /necropsy rooms or in approved investigator laboratories.

The ARAC Guidelines: Guidelines for Euthanasia of Rodents Using Carbon Dioxide, and Guidelines for the Euthanasia of Rodent Feti and Neonates are resources for use by veterinary staff members, investigative staff, and ACUC members.

CO_2 euthanasia is the most common method used for rodents at NIEHS. An SOP developed by CMB veterinarians and approved by the ACUC is available to all investigators. CO_2 lines with flow meters, and instructions for use are provided in all procedure rooms and laboratories where CO_2 euthanasia of rodents is performed. A carbon dioxide manifold system and SOP has been designed as a refinement for controlled delivery of CO_2 to animals in home cages. This offers an efficient reduced stress method of CO_2 euthanasia appropriate for all ages of rodents and is used by trained animal husbandry personnel. A secondary method is required to ensure death.

Investigators approved for cervical dislocation and decapitation in unanaesthetized mice and rats must be evaluated for proficiency by the VMS.

NIEHS also has an ACUC approved guideline for the euthanasia of rodent fetuses and neonates.

Approved Methods of Euthanasia:

Mice
- CO_2 –via regulated source (flowmeter)
- Cervical dislocation with/without anesthesia
- Decapitation with/without anesthesia
- Overdose of sodium pentobarbital
- Overdose of inhalant, isoflurane
- Exsanguination or perfusion under deep anesthesia

Rats
- CO_2 – via regulated source (flowmeter)
- Decapitation with/without anesthesia
- Overdose of sodium pentobarbital
- Overdose of inhalant, isoflurane
- Exsanguination or perfusion under deep anesthesia

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

Flowmeters for CO_2 are installed and maintained by ORF. Appropriate flow rates are determined by VMS and posted by each flowmeter. There is an SOP for CO2 euthanasia.

Scissors and guillotines should be tested prior to each use and can be tested on a routine basis for sharpness by cutting a thick (2-3 mm) rubber band—an appropriately sharp blade will cut without dragging the rubber band. Only sharp scissors should be used and scissors must be replaced as needed. When a guillotine must be sharpened VMS will be responsible for getting the guillotine serviced or replaced. If a guillotine is needed during the time of service, one will be loaned out through VMS. There is an SOP for the maintenance of sharps used for decapitation.

Equipment used for euthanasia is checked during the semiannual site visits by the ACUC.

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

Death in rodents is confirmed by decapitation, cervical dislocation (mice only), or thoracotomy.

IV. Physical Plant [Guide, pp. 133-151]

Repeat this section for each animal housing area, including agricultural settings, temporary holding areas for field studies, aquatic environments, and each IACUC/OB approved satellite housing facility. Include as an appendix the floor plans of each (if applicable) on 8.5" x 11" or A4 paper.

A. Location and Construction Guidelines

1. Note the location (building, floor, wing, etc.) of the animal facility(ies). Describe the management structure and program oversight for each of the areas listed in this section.

The NIEHS animal facilities are located on the South Campus, Redacted by agreement Redacted by agreement Animal feed and bedding is received and stored in the building Redacted by agreement warehouse and is transported and sterilized in Redacted by agreement (Animal Bedding and Feed). Sterilized feed and bedding is stored and dispensed in room Redacted by agreement (clean side of Animal Bedding and Feed).

CMB offices and laboratories are located on the Redacted by agreement

CMB provides management, programmatic and administrative oversight for the animal facilities. The CMB ARS Program Manager (Contract Officer Representative) and CMB ARS Facility Manager oversee all animal facility operations and have offices located in Redacted by agreement animal facility. The contractor provides an onsite Project Manager, Assistant Project Manager as well as area supervisors who have offices located in the animal facility.

2. Describe the physical relationship of the animal facilities to the research laboratories where animals may be used.

Research laboratories are in Redacted by agreement and are located on the upper floors Redacted by agreement Redacted by agreement

3. Describe the general arrangement of the animal facilities (e.g., conventional, clean/dirty corridor, etc.). For animals that are maintained in a laboratory in order to satisfy the scientific aims of a protocol, describe the housing and care provided and the maximum period of stay required.

The NIEHS animal facility is managed as an SPF facility. The facilities are operated on a clean/return corridor concept. Incoming animals and equipment are received at a centralized loading dock and are transported to animal rooms or storage areas in the animal facility via the return corridor. Laboratory and engineering support personnel enter, exit, and access animal rooms via the return corridor after donning appropriate PPE. Animal care personnel may enter animal rooms via the return corridor or via the clean corridor using procedures outlined previously. Clean equipment is supplied to animal rooms via the clean corridor. Soiled/used equipment is returned to the cage wash areas via the return corridor. Redacted by agreement share a common cage wash area; Redacted by agreement has a separate cage wash facility.

Animals are not housed in laboratories, but may be transported to laboratories for holding less than 24 hours. Animals may not return to the animal facility.

4. Describe finishes throughout the animal facility (ies) for floors, walls, ceilings, doors, alleyways, and gates. Note any areas that are not easily sanitized and describe how these areas are maintained.

Corridors are 8 feet wide. Corridor walls are constructed of epoxy painted cinderblock, protected by cove base. Corridor and animal room floors are constructed of concrete and are covered with sealed epoxy.

Walls in most animal rooms and in all cage wash areas are constructed of sealed cinderblock and covered with epoxy paint. Walls in radio frequency shielded (lead lined) animal rooms designed for electrophysiology studies are comprised of plaster.

Animal Room Doors are sealed metal, 7'9" high and 4' wide with viewing panels composed of single pane glass.

Ceilings of corridors, cage wash areas and most animal rooms are constructed of concrete covered with epoxy paint. Ceilings in radio frequency shielded (lead lined) animal rooms designed for electrophysiology studies are comprised of plaster.

5. If exterior windows are present within the animal housing or procedure areas, describe IACUC/OB consideration regarding temperature and photoperiod control, as well as potential security risks.

There are no exterior windows present within the animal housing or procedure areas.

B. Functional Areas and Operations

1. Heating, Ventilation, and Air-Conditioning (HVAC) [Guide, pp. 139-140, 143]

a. Describe the mechanical systems used to provide temperature, humidity and air pressure control. Include details such as the use of variable air volume (VAV) systems, and additional key features of HVAC systems affecting performance.

The heating, ventilation and air conditioning systems for the animal facilities are 100% outdoor air units. The animal facility units are separate from the rest of the building. One hundred percent outdoor air is filtered and conditioned in 3 separate central air handling units for both supply and exhaust. Redacted by agreement of the Animal Bedding and Feed (ABF) building. These units receive 100% outdoor air from louvers located along the exterior wall of the building between Redacted by agreement modules. The Redacted by agreement module units are in the Redacted by agreement Redacted by agreement The louver locations prevent entrapment of fumes from vehicles or building exhaust. Each module operates independently from the other modules and each have a separate redundant standby system. Key components of all supply and exhaust systems include fans, pre-heat coils, cooling coils, humidifiers (at the units and the individual rooms), and isolation dampers. Re-heat coils and humidifiers are located at each individual room to provide greater control of temperatures and humidity. All 100% outdoor air is HEPA filtered prior to entering the HVAC system.

Temperature and humidity sensors are wall mounted in each animal room as well as located in the exhaust duct of each animal room. Room temperatures and humidity are programmed and monitored on a twenty-four-hour basis through the Metasys Building Automation System. If either temperature or humidity exceeds the high or low limit set points an alarm condition will be indicated in the Building Engineers Office Redacted by agreement and

BAS automatically adjusts to bring the parameter back into the acceptable range. Alarm response is staffed 24 hours a day. Any abnormalities are immediately checked and corrective actions are taken.

The features of a typical animal holding room are as follows;

1) Air is supplied through laminar flow type diffusers located in the ceiling. Air is exhausted through low sidewall registers located near the floor in two corners of the room.

2) In Illinois cubicles, air enters thru the lower portion of each cubicle door and is exhausted thru the ceiling of each cubicle.

3) Room temperature and humidity is controlled by a thermostat and humidistat located in the animal room. The thermostat and humidistat in the exhaust air duct is used for verification.

4) Temperature and humidity is controlled at the room level and can be maintained anywhere from a lower design condition of 65 °F dry bulb and 50% relative humidity to an upper design limit of 80 °F dry bulb and 50% relative humidity. Animal room temperature set points are 72 +/- 2°F.

5) Room ventilation rate is greater than 10 air changes per hour.

6) Supply and exhaust air quantities are controlled at a constant preset rate by an air flow monitoring and control device located in the room air supply and exhaust ducts. Each conventional animal holding room is maintained at a negative pressure relative to the clean corridor and positive to the dirty corridor. Containment rooms are maintained at a negative pressure relative to both corridors.

7) The exhaust from conventional animal rooms is filtered through high fiber filters. Rooms that may be used for containment purposes Redacted by agreement Redacted by agreement Illinois cubicle room Redacted by agreement procedure room Redacted by agreement and surgical suite room Redacted by agreement are exhausted through HEPA and carbon filters.

b. Describe construction 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.

All animal room reheat coils fail to the closed position upon high temperature alarm. Humidification steam coils are equipped with a high limit shut off to prevent space overheating with valve failure.

c. Describe how critical air pressures, ventilation, and temperature are monitored and maintained in the event of a system or component failure.

Supply air and exhaust air quantities are controlled at a constant preset rate by air flow monitoring and control devices located in the room air supply and exhaust ducts. In the event of a supply air system failure, the exhaust fan modulates to a lower or off position to prevent the animal rooms from drawing air from the return corridors. If the primary air supply unit shuts down, the stand-by unit is activated. If the exhaust system malfunctions, the stand-by unit is activated. In each of these scenarios the affected animal area pressurization is verified through the Metasys system.

d. Describe procedures for monitoring animal facility mechanical systems and notifying appropriate personnel in the event of a significant failure that occurs outside regular work hours.

Animal facility mechanical systems are monitored through the Metasys Building Automation System which monitors operation of the Air Handling Units as well as room temperature and humidity levels. In the event of a failure, an alarm is sent, via Metasys, to the Building Engineers Office (which is operated 24/7). Alarm parameters are set for both a high and low limit. Notifications are also sent via e-mail to ORF contacts on a 24/7 basis. The Building Engineer maintains an emergency contact list of personnel to be notified when issues arise that require immediate/emergency response.

Air supply to the semi-rigid isolators is maintained on emergency power circuits, as well as battery back-up units (maintain air pressure for up to 20 hours [last tested February 2014]). Each isolator is also equipped with an air pressure alarm wired to the Metasys system.

2. Power and Lighting [Guide, p. 141]

a. Note if emergency power is provided for the animal facility and if so, what electrical services and equipment are maintained in the event the primary power source fails.

Emergency power is supplied by two (2) diesel generators with a capacity of 1800 KW which automatically start and provide power whenever commercial power is interrupted for more than five (5) seconds. The fuel capacity of the generators will sustain power for 6.9 days. All animal area air supply and exhaust systems are connected to the emergency power system. Emergency generators will continue to supply power to provide the necessary electricity for critical services; ventilation controls and monitoring, ventilated caging systems, gnotobiotic isolators, surgical areas, freezers, incubators, designated emergency outlets, and lighting throughout the animal facility.

b. Give history of power failures for the animal facility. Note frequency and duration. If emergency power was not available during a power failure, describe steps taken to ensure the comfort and well-being of the animals and the temperature extremes reached in the animal rooms during the failure.

Since 2014 there has been one power failure which was immediately responded to by the Office of Facilities Management. The emergency generator engaged and power to the animal facility was restored immediately. Temperature and humidity rose in the hallway Redacted by agreement but disapated within 5 minutes of the failure. Animal rooms were not affected.

c. Describe lighting system(s) for the animal housing facility(ies). For each species or holding room type, list light intensity, photoperiod (Light:Dark), construction features (e.g., water resistance), and control (e.g., automatic versus manual, phasing). For systems automatically controlling photoperiod, describe override mechanisms.

Water proof electronic ballast T8 light fixtures are surface mounted and automatically controlled through the Metasys system. In general, light cycles are set for 12 hours light/12 hours dark. Metasys permits the program to vary from room to room and to be changed if required by program needs.

Three level lighting controls are provided for each animal holding room; intensity is adjustable and the routine illumination level is set at 35-40 foot candles. The intensity may be increased if necessary for the performance of certain duties in the animal room. Lighting control switches are in the return/clean corridors and are encased to provide water resistance. The override switch permits manual operation of lights locally when the lights are off. The switch is a timer type that permits "ON" operation of the lights for up to 60 minutes, after which, the timer turns the lights "OFF" automatically.

Location of the override switches are as follows:

Redacted by agreement

Overhead corridor lighting is programmed from the Metasys system. There are no wall switches and overhead lighting will be either all "ON" or all "OFF" as programmed. Corridor lights come "ON" 30 minutes before the animal room lights in the morning and go "OFF" 30 minutes after the animal room lights in the evening. To permit the use of the corridors while overhead lighting is "OFF" and to provide emergency egress lighting, there are recessed wall mounted louver faced aisle lights located approximately 18 inches above the floor in all corridors. These lights provide a very low intensity light level.

Redacted by agreement is a multipurpose animal room that can be used for special light studies and has manual lighting controls. Room Redacted by agreement is monitored through Metasys, but is controlled (on/off) through the time clock in the corridor just outside of the animal room.

Note: For behavioral testing purposes, Redacted by agreement is currently on a reverse light cycle (lights are on between 8PM and 8AM).

If animal room lighting does not come on or shut off at the designated time, an alarm is sent through the Metasys system to the Building Engineers office. The operator on duty investigates and makes the correction. If lighting malfunctions, a manual switch can be overridden in the Metasys system. The Building Engineers maintain a log book documenting any malfunctions and corrective actions. A recheck of the Metasys components is completed the day following any failure to confirm proper functioning. Reports from the Metasys system can be generated which will show all alarms.

3. **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)

No adverse effects in any animal population have resulted from system malfunctions.

4. **Storage Areas** [Guide, pp. 141-142]

   a. Describe storage areas for feed and bedding, including temperature and vermin control.

The NIEHS Redacted by agreement warehouse on south campus houses 1284 sq foot climate controlled (55 to 65 ° F/50% RH) bedding and feed storage room with independent environmental controls. This room is monitored and controlled through the Metasys system.

Bulk shipments of feed are ordered every 8 weeks and the feed is stored on pallets, which are placed on metal racks. At weekly intervals, small batches (3-4 pallets) of feed are transported to the Redacted by agreement Animal Bedding and Feed (ABF) area where they are stored in Room Redacted by agreement an air-conditioned room (temperatures 55-65°F), until autoclaved. After autoclaving, feed is stored for at least 24 hours to cool in Room Redacted by agreement (clean side of ABF) at 55-65 °F before it is transferred manually or via the Ducon pneumatic feed/bedding dispensing system to the dispensing sites for Redacted by agreement Redacted by agreement or to the dispensing site for Redacted by agreement Clean Cage Wash Area). See section II.C.vii for further information.

Bulk shipments of bedding are ordered every 3 to 4 months and stored on pallets, which are placed on metal racks. At weekly intervals, small batches (4-5 pallets) are transported to the Redacted by agreement ABF area for autoclaving. After autoclaving, bedding is stored for at least 24 hours to cool in Redacted by agreement (clean side of ABF) at 55-65 °F before transferred manually or via the Ducon pneumatic feed/bedding dispensing system to the dispensing sites for Redacted by agreement or to the dispensing site for Redacted by agreement

Redacted by agreement ABF Area consists of two (2) autoclaves to process bedding and feed for sterilization. Both the clean and return areas of ABF are controlled and monitored through the Metasys system.

The enclosed feed and bedding holding area is monitored daily with live traps supplied with Napa Nectar™ by Animal Husbandry Support Contract Staff. Pest monitoring stations are used to monitor for cockroaches, ants, and silverfish, etc. and are replaced monthly or more often as needed.

Storage in Animal Rooms

Feed in the animal rooms is stored in Rubbermaid bins. Feed cards are attached to the side of the bin and list feed type, chute #, mill date, date placed in room, and expiration date. Each animal room contains one live trap and one insect monitoring station. Additionally, Illinois cubicle rooms contain one insect monitoring station per cubicle. Animal technicians check traps daily and findings are logged on the "Pest Control Form". Bedding is not stored in animal rooms except for those studies using paper bedding which may be stored in a labeled microisolator covered sanitized cage or Rubbermaid bin.

b. Describe storage areas for cages, equipment, supplies, etc.

Clean caging and equipment is stored in the marshalling areas of the clean cage wash rooms Redacted by agreement as well as along the clean corridors. New equipment and supplies are stored in designated storage areas in Modules Redacted by agreement Redacted by agreement These rooms are outfitted with stainless steel shelving and plastic pallets.

c. Describe storage areas for flammable or hazardous agents and materials (e.g., disinfectants, pesticides, fuel).

Flammable agents are stored in vented cabinets in Redacted by agreement (isolator cylinder preparation room). The location and type of cabinet is approved and inspected by the NIEHS HSB.

Limited quantities of materials being used in a study are stored in a dedicated, properly identified refrigerator in Redacted by agreement The agents are returned to the investigator after completion of the study. Treated feed is stored in a dedicated refrigerator in Redacted by agreement Redacted by agreement or in a dedicated cold room in Redacted by agreement

5. Facilities for Sanitizing Materials [Guide, pp. 153.]

Describe for each cage sanitation area its location, the traffic flow pattern (soiled to clean, or in and out) within the facility, and kinds of equipment (tunnel washer, bottle washer, rack washer, etc. and other related equipment such as bedding dispensing units).

Cage wash facilities are in Room Redacted by agreement Redacted by agreement Cage wash areas are operated on a clean/return corridor concept and are accessed via automatic doors. All new or dirty cages, equipment and accessories are transported to the soiled side of the cage wash area via the return corridor. Accessories are sorted and put in baskets; cages and equipment are dismantled, emptied, and washed in tunnel type cage, rack, or bottle washers. All clean cages, racks, equipment, and accessories are inspected, assembled, stored, or distributed in or from the clean side of the cage wash area. Bedding is automatically dispensed into the cages prior to delivery to the animal rooms via the clean corridor.

Sanitation equipment consists of three tunnel type cage washers, two tunnel type bottle washers, and two cabinet style rack washers. Each clean cage wash area is equipped with an automated feed and bedding delivery/dispenser system (Ducon) and bottle filler. Each soiled cage wash area is equipped with a hood/vented dump station and an automated waste removal (Ducon) system. All washers are operated at 160-180° F, with varying pre-wash, wash, rinse, and final rinse cycles. Supplemental exhaust ventilation is provided for the rack washers. Cage wash areas have sinks for soaking and hand washing miscellaneous equipment. In addition, Module E cage wash area is equipped with a steam autoclave.

Sound attenuation panels and eyewash stations are installed in each cage wash area.

C. Special Facilities [Guide, pp. 144-146, 150]

1. Specialized Types of Animal Housing

Note specialized types of available animal housing spaces such as barrier, hazard containment (infectious, radioactive, chemical), "animal cubicles" (also known as "Illinois Cubicles", "Horsfal Cubicles," and "animal modules"), or facilities designed specifically for housing certain species such as aquatic or agricultural animals (e.g., barns, feedlots). [Guide, pp. 160-161]

Semi-rigid isolators house mice in Redacted by agreement

Specialized housing space for studies using hazardous agents is available in all three modules of the animal facility (Illinois cubicles in Redacted by agreement containment rooms in Redacted by agreement

Redacted by agreement Ventilated cage racks can provide positive or negative airflow containment. These racks are fitted with stand-alone supply/exhaust air and can be used in any animal room.

The TSE Labmaster system, designed to measure metabolic performance and food and water consumption in mice is used in room Redacted by agreement This system is composed of solid bottom, bedded, plexiglass cages. The specially designed cage lid contains connections for fresh air, sampling, and air outlet. The lid also contains three additional openings for drinking water, feed container, and temperature sensors.

2. **Surgery** [Guide, pp. 144-145]

   a. Describe facilities for aseptic surgery, surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery.

Survival surgery is performed in the animal facility:
Surgical Suites- Redacted by agreement
Procedure Rooms- Redacted by agreement
Redacted by agreement used by the NIEHS knock-out core for embryo transfer/NSET.

Support areas include surgeon prep area, instrument prep area, animal prep area, post-operative recovery area, storage area for oxygen and supplies, and an autoclave room.

   b. Describe construction features of the operating room(s), including interior surfaces, ventilation, lighting, and fixed equipment used to support surgical procedures and enhance contamination control.

Walls and ceiling are constructed of epoxy painted cinder block. Floors are constructed of epoxy coated sealed concrete. Ventilation systems provide filtered air at positive pressure relative to the return corridor. Recessed fluorescent lights are complimented by ceiling mounted surgery lamps located above the surgery tables. Floors and surfaces are cleaned daily. Surgical scopes maintained in the surgery rooms are kept covered.

3. **Other Specialized Animal Use Facilities** [Guide, pp. 146-150]
Describe other facilities such as imaging, irradiation, and core behavioral laboratories or rooms. Include a description of decontamination and methods for preventing cross-contamination in multi-species facilities.

Behavior testing is performed in rooms Redacted by agreement Imaging is performed in rooms Redacted by agreement Work with radioisotopes is performed in Redacted by agreement Walls and ceilings are constructed of epoxy painted cinder block. Floors are constructed of epoxy coated sealed concrete. Ventilation systems provide filtered air and relative pressure is set based upon room usage.

Walls and ceilings of the cesium irradiation room Redacted by agreement are constructed of epoxy painted cinder block. Flooring is vinyl. Access is restricted by the HSB. An SOP is in place for the use of this room and exposure of animals.

SOPs are in place for specialized equipment which includes procedures for sanitation. All instrumentation in the imaging room is kept covered and areas are cleaned and decontaminated after use.

4. Other Animal Support Facilities

Describe other facilities providing animal care and use support, such as food preparation areas, feedmills, abattoirs, etc.

Redacted by agreement Feed Prep Room, is equipped with a counter, sinks, feed grinder, and exhaust line and can be used by the QAL to prepare small quantities of special diets (non-chemically treated).

The Diet Kitchen Redacted by agreement is used to prepare isolator cylinders prior to sterilization and transfer of supplies to the flexible film isolators.

D. Security and Access Control [Guide, p. 151]

Describe such features as control of entry, perimeter fences, gates, entryways, cameras, guards.

The NIEHS campus employs Redacted by agreement

Redacted by agreement

The NIEHS campus main entrance is Redacted by agreement

Redacted by agreement

The electronic access control system Redacted by agreement

Redacted by agreement

Redacted by agreement

Appendix 1

NIH Office of the Director

OD, OIR, Office of Animal Care and Use

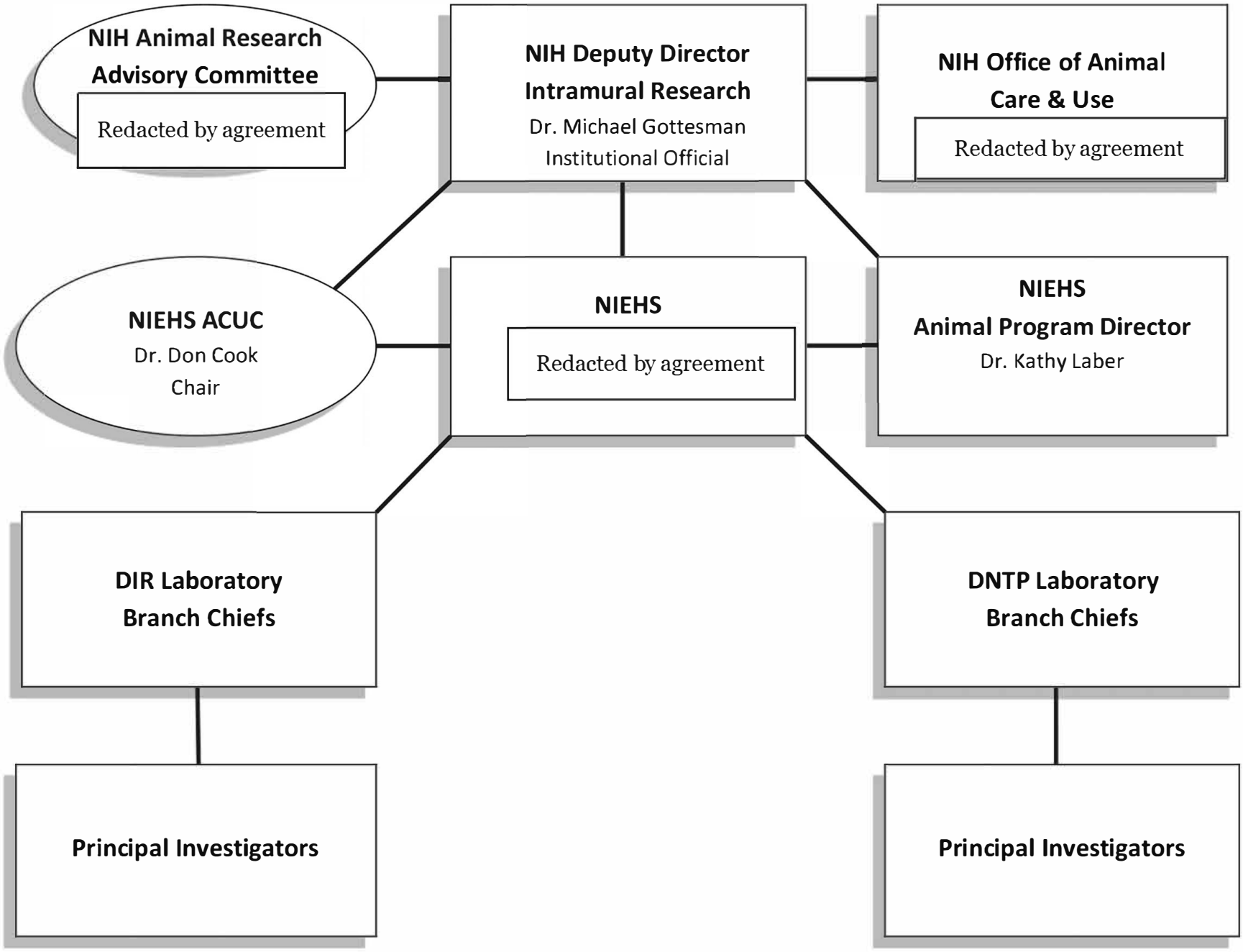
Institutes/Centers Using Animals in Research

- OD, DCPSI, Office of Research Infrastructure Programs
- OD, OM, ORS, Division of Veterinary Resources (DVR)
- National Cancer Institute (NCI)
- National Eye Institute (NEI)
- National Heart, Lung and Blood Institute (NHLBI)
- National Human Genome Research Institute (NHGRI)
- National Institute on Aging (NIA)
- National Institute on Alcohol Abuse and Alcoholism (NIAAA)
- National Institute of Allergy and Infectious Diseases (NIAID); DIR, DCR, RML, VRC
- National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- National Institute of Biomedical Imaging and Bioengineering (NIBIB)
- National Institute of Child Health and Human Development (NICHD)
- National Institute on Deafness and Other Communicative Disorders (NIDCD)
- National Institute of Dental and Craniofacial Research (NIDCR)
- National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- National Institute on Drug Abuse (NIDA)
- National Institute of Environmental Health Sciences (NIEHS)
- National Institute of Mental Health (NIMH)
- National Institute of Neurological Disorders and Stroke (NINDS)
- NIH Clinical Center (CC)
- National Center for Advancing Translational Sciences (NCATS)
- National Center for Complementary and Alternative Medicine (NCCAM)

Institutes/Centers With No Animal Research

- National Institute of General Medical Sciences (NIGMS)
- National Institute of Nursing Research (NINR)
- National Library of Medicine (NLM)
- National Institute on Minority Health and Health Disparities (NIMHD)
- Fogarty International Center (FIC)
- Center for Information Technology (CIT)
- Center for Scientific Review (CSR)

NIEHS IACUC Diagram



2017 NIEHS Division of Intramural Research Organizational Chart

Redacted by agreement

Appendix 4

Updated: July 2017

COMPARATIVE MEDICINE BRANCH

BRANCH CHIEF/ATTENDING VETERINARIAN
Kathy Laber, DVM, DACLAM

Redacted by agreement

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Developmental Pharmacogenetics.	Redacted by agreement		Mouse	1983	C, D, E	x		x		x	x
Glucocorticoids Block Estrogen-Induced Uterine Growth.			Mouse	937	C & D	x				x	x
Activity-Dependent Regulation of Neuronal Gene Expression.			Mouse Rat	Mouse - 3590 Rat - 140	C & D						x
Characterization of COX Mice.			Mouse	9434	C & D						x
Analysis of Estrogen Hormone Mechanisms and Responses in ERKO mice.			Mouse	181902	C & D	x	x			x	x
Investigation of Mechanisms Underlying Bleomycin-Induced Pulmonary Fibrosis.			Mouse	1920	C & D					x	
Role of Inflammatory and Anti-Inflammatory Mediators in Ozone-induced Lung Injury.			Mouse	1440	C						
Role of Eicosanoids in the Cardiovascular System.			Mouse	2415	D & E	x					x
Breeding Protocol for Analysis of Hormone Signaling in Mice Deficient in Histone Subtypes and Histone Modifying Enzymes.			Mouse	2265	C						x
Role of Nrf2 and Antioxidant Proeteins in Lung Carcinogenesis.			Mouse	2480	C & D					x	
Gene Knockout mice Breeding Colonies.			Mouse	8712	C						
Nrf2 Gene Knockout and Wild-type Mouse Breeding Colonies.			Mouse	11353	C & D						
Role of P450 Products in Vascular Inflammation.			Mouse	1932	C, D & E	x				x	
Breeding of Genetically Engineered Mice for Experimental Analysis.			Mouse	28120	C & D						
Chemokine and Toll-like Receptor Adaptor Function in Pulmonary Inflammation.			Mouse	38240	C, D, & E	x				x	x
Physiological Function of Glis Proteins.			Mouse	26913	C, D & E			x		x	x
Study of the Function of the Nuclear ROR Receptors.			Mouse	2200	C, D & E						x

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Physiological Function of TAK1 Receptors.	Redacted by agreement		Mouse	1275	C & D					x	x
NAG-1 Transgenic Mice.			Mouse	170	C						
Host Resistance to Viral and Bacterial Infections in Multiple Strains of Mice.			Mouse	7030	C					x	x
Glucocorticoid Receptors - Inflammation, Endotoxemia, and Sexual Dimorphism.			Mouse	1791	C, D & E	x				x	x
The Role of Lipids in Pulmonary Innate Immunity.			Mouse	63658	C, D & E					x	x
Necropsy and Tissue Collection for CMPB and NIEHS Investigators.			Mouse Rat	Mouse - 432 Rat - 150	C & D						x
Glucocorticoid Receptor Isoforms Knock-In Mice.			Mouse	5284	C, D & E	x					x
Role of Cholesterol Trafficking in Lung Inflammation.			Mouse	48118	C						
Hyperoxia-induced Injury in Developing Mouse Lungs.			Mouse	3820	C					x	
Sir2 and Nuclear Receptors in Aging and Age-associated Diseases.			Mouse	2216	C, D & E			x		x	x
Sir2 and Nuclear Receptors in Aging and Age-associated Diseases.			Mouse	4342	C						
Role of Glucocorticoid Receptor and Hes1 Signaling in the Mouse Eye.			Mouse	5760	C & D	x				x	x
The Role of Inter-alpha-trypsin Inhibitor in Wound Healing and Angiogenesis after Injury.			Mouse	22600	C & D	x				x	x
Breeding Colony for Transgenic Experimental Strains.			Mouse	25293	C & D						x
The Role of Inter-alpha-trypsin Inhibitor in Complement Dependent Inflammation.			Mouse	3384	C, D & E						x
Mechanisms of Fertilization.			Mouse	12551	C, D & E					x	x

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Effects of Estrogen in Differentiation.	Redacted by agreement		Mouse	13308	C & D	x				x	x
The Role of Hyaluronan Binding in Airway Hyperreactivity in Asthma.			Mouse	42524	C & D					x	x
Examination of Free Radicals In Vivo and In Vitro.			Rat	66	C & D					x	
Glucocorticoid Receptor Phosphorylation Site Knock-In Mice.			Mouse	2225	C						
Production and Breeding of KO/TG Mice in the Knock Out Core .			Mouse	24711	C & D	x					x
Receptor Mechanisms in Hormone-Sensitive Cells Derived from CRACM1 Knockout Mice.			Mouse	12752	C, D & E						x
Conditional Glucocorticoid Receptor Knockout in Mouse Uterus.			Mouse	3296	C & D	x				x	x
Breeding Protocol for Conditional Glucocorticoid Receptor Knock-out Mice.			Mouse	40172	C, D & E					x	x
Generation and Maintenance of Developmental Pharmacogenetics Breeding Colonies.			Mouse	24777	C					x	x
Functional Assessment of Hippocampal Area CA2.			Mouse	3548	C, D & E	x	x	x		x	x
Pluripotentcy Factors in Development and Disease.			Mouse	26990	C & E					x	x
Use of Transgenic Mice to Study Noradrenergic Neuron Development, Diversity, and Function.			Mouse	21745	C, D & E	x	x	x	x	x	x
Investigation of Mechanisms Underlying Respiratory Syncytial Virus Disease.			Mouse	2880	C					x	

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Evaluation of Hippocampal Neural Progenitor Cells and Altered Neuroimmune Regulation as Influenced by Arsenic Exposure.	Redacted by agreement		Mouse	Mouse - 1710	C & D					x	x
Identifying the Process of Reproductive Organ Formation.			Mouse	268476	C & D	x		x		x	x
Conditional Glucocorticoid Receptor Knockout in Murine B Cells.			Mouse	1803	C						x
Characterization of Stathmin 1 in Skin Carcinogenesis: Breeding of Stathmin Deficient Mice.			Mouse	5026	C						
Development and Refinement of Analgesics in Laboratory Animals.			Mouse	Mouse - 198 Rat - 174	C & D	x					x
Physiological Function of JAZF1/TIP27.			Mouse	10308	C & D					x	x
Physiological Function of RORa.			Mouse	2892	C & D						x
Genetic Manipulation of Hippocampal CA2 Neurons.			Mouse	40983	C, D & E	x				x	x
Mouse Assisted Reproductive Services - Rederivation, Cryopreservation and Embryo Transfer.			Mouse	1476	C & D	x					
Genetic Analysis of DNA Methyltransferases (DNMTs).			Mouse	3771	C						x
In Vivo Electrophysiological and Behavioral Analyses of CA2 Function.			Mouse	Rat - 438	C & D	x		x			x
Physiological Function of RORg.			Mouse	121	C & D					x	
Breeding Protocol for GWAS Candidate Genes in Lung Function			Mouse	12773	C						
Role of GWAS Candidate Genes in Lung Function.			Mouse	2874	C & D					x	x
NIEHS Research Animal Health Surveillance Program.			Mouse	Mouse - 8270 Rat - 168	C						x
Protein Radical Formation in Maneb and Paraquat Induced Parkinson's Disease.			Mouse	224	C, D & E					x	x

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Viral Deliver of Genes to Single Cell Embryos.	Redacted by agreement		Mouse	2763	C & D	x				x	
Metabolism and Disposition of Tetrabromobisphenol A (TBBPA) in Female Wistar-Han Rats.			Mouse	316	C						x
Physiological Effects of Natural or Contaminating Compounds in a Rodent's Microenvironment.			Mouse	2019	C					x	x
Embryoninc Stem Cell Evaluation.			Mouse	399	C					x	
Experimental Protocol for Analysis of Hormone Signaling in Mice Deficient in Histone Subtypes and Histone Modifying Enzymes.			Mouse	252	C					x	x
Metabolism and Disposition Studies of Brominated Components of a Commercial Flame Retardant.			Mouse	Mouse - 672 Rat - 864	C	x				x	x
Primary Murine Spermatogonial Germ Cell Culture.			Mouse	4048	C & D						
Measurements of Neuronal Activity In Vivo.			Mouse	39	D & E	x					x
The Role of Inter-alpha-trypsin Inhibitor in Obesity and Obesity-related Diseases.			Mouse	6028	C						x
Characterization of Breast Cancer Cells that Express GATA3 Mutants			Mouse	540	D	x	x			x	
Role of FeSOD2 in Iron Toxicity: Environmental Exposure to Iron and Genetic Susceptibility.			Mouse	252	C & D					x	x
Regulation and Function of Mineralocorticoid Receptors in Hippocampal CA2 Neurons.			Mouse	2636	C, D, & E	x	x		x	x	x
Role of Colinergic Signaling in Brain Function: Use of Transgenic Mouse Models and Viral Vectors.			Mouse	1626	D	x	x			x	x
Physiological Function of Glis3 Protein.			Mouse	3240	C						x
Role of SIRT1 in Intestinal Stress Response and Inflammation.			Mouse	224	C, D & E					x	

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Effects of Hyaluronan Functionalized Multi-walled Carbon Nanotubes in C57BL/6 Mice.	Redacted by agreement		Mouse	1056	C					x	
Epigenetic Changes in Mouse Intestine in Response to Diet.			Mouse	112	C					x	x
Breeding and Maintenance Protocol for Studies of LC3-associated Phagocytosis as a Critical Regulator of Inflammation.			Mouse	6604	C						
The Role of Acetylcholine in Learning and Memory Function in Rodents.			Mouse	570	C & D					x	x
Determining the Role of the Locus Coeruleus in Neurodegeneration.			Mouse	460	D	x				x	
Conditional Glucocorticoid Receptor Adipose Tissue Knock-out Model.			Mouse	756	C						x
Conditional Glucocorticoid Receptor Knock-out in the Heart.			Mouse	976	D & E	x				x	x
Conditional Glucocorticoid Receptor Knock-out in Mouse Liver.			Mouse	720	C, D & E	x				x	x
Conditional Glucocorticoid Receptor Knockout in Mouse Brain.			Mouse	2232	C & D	x					x
Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders. (Breeding protocol)			Mouse	9352	C & D						
Do Physiologic Parameters of Mice Vary with Caging Type?			Mouse	336	C & E						
Tissue and SampleHarvest for the Study of LC3-associated Phagocytosis.			Mouse	11574	C						x
The Role of LC3-associated Phagocytosis in the Development of Diabetes Mellitus.			Mouse	9280	D					x	
The Role of LC3-associated Phagocytosis in Autoimmune and Inflammatory Disorders.			Mouse	17592	D					x	

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Perinatal Exposure to Tetrabromobisphenol-A and Latent Health Effects in Rats.	Redacted by agreement		Mouse	763	C					x	
Clinical Evaluation of LLLT, Buprenorphine, Chlorhexidine in Clinical Disease.			Mouse	132	C & E					x	x
DeMayo Mouse Breeding Protocol.			Mouse	9216	C & D						x
Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders			Mouse	6264	C & D	x	x	x		x	x
The Role of LC3-associated Phagocytosis in Arthritis and Colitis Models.			Mouse	11096	D & E					x	
Behavioral Studies in Mice.			Mouse	312	C & E						x
Repetitive Element and Mitochondrial Metabolism in Epigenetic Control.			Mouse	1610	C & D					x	
The Role of LC3-associated Phagocytosis in Neuroinflammatory and Neurodegenerative Disorders.			Mouse	2400	D & E	x				x	
Generation, Initial Characterization and Maintenance of Pgc1a SINE KO Mice.			Mouse	792	C & D						x
Isolation and Characterization of Stem Cells and Cancer Stem Cells.			Mouse		C, D, & E	x				x	
The Role of LC3-associated Phagocytosis in Bacterial and Fungal Infection.			Mouse	3264	D & E					x	
Cadmium Positive Control Study.			Mouse	10	C					x	
DeMayo Reproductive Biology Investigations.			Mouse	10101	C & D	x	x			x	x
Developmental Neurotoxicity of Fluoride.			Mouse	3270	C, D, & E						
Dopamine Neurons Derived from Human ES Cells Efficiently Engraft in Animal models of Parkinson's Disease.			Mouse	100	D	x	x			x	x

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
In Vivo Study of the Role of Acetylcholine in Learning and Memory.	Redacted by agreement		Mouse	540	D	x	x			x	x
Transcriptomic Signatures of Developmental Endocrine Disruption Chemical (EDC) Exposures.			Mouse	946	C					x	
The Role of Tlr5 in Inflammatory and Fibrotic Disease.			Mouse	3288	C, D, & E					x	x
The Role of Steroid Hormones in Gastric Function.			Mouse	1717	C & D	x	x			x	x
Differentiation and Transplantation of Pancreatic Beta- like Cells Derived from Human Embryonic Stem Cells.			Mouse	80	D	x				x	x
Genetic Analysis of MTAs.			Mouse	8199	C						x
Maintenance of NIEHS-generated, Complex Genetically Modified Mouse Models.			Mouse	4710	C & D						
Knockout of Metastasis-associated Genes in Mouse Intestine.			Mouse	2160	C						
Metabolism and Disposition Studies of 2,4,6 Tribromophenol (TBP).			Mouse	Mouse - 184 Rat - 224	C	x				x	x
Role of Long Non-coding RNA G3R1.			Mouse	1315	C & D			x		x	x
Molecular Analysis of Dopamine Neuron Loss in Mouse Models of Parkinson's Disease			Mouse	200	D	x	x			x	x
Adult Neurogenesis in the Mouse Brain.			Mouse	200	C & D					x	x
DeMayo Group Lung Research Protocol.			Mouse	602	C						x
Evaluating the Neurotoxicity of Hexachlorophene in Rats Using Magnetic Resonance Microscopy (MRM).			Mouse	20	D					x	x

Protocol Title	IACUC/OB No.	Principal Investigator	Species	# of Animals Approved	Pain and Distress Category*	SS	MSS	FFR	PR	HAU	NCF
Mouse Models of Arhinia and Other Rare Human Syndromes (Mouse Colony Breeding Protocol).	Redacted by agreement		Mouse	880	C, D, & E						x
Pilot Study of Sex Differences in Neurobehavioral Tests.			Mouse	282	E						
The Role of SIRT1 in Mouse Development.			Mouse	7774	C & E					x	x
MBD3 Mutant Mouse Generation.			Mouse	2482	C						
The Role of Hyaluronan Associated Proteins in Tobacco-induced Inflammation.			Mouse	1120	C & D					x	x
Training and Development of Technical and Surgical Procedures, Analgesic and Anesthetic Protocols.			Mouse Rat	Mice - 1580 Rats - 810	C & D	x					x
Roles of Brain Neuroimmune Function in Inflammation-Related Neurodegeneration.			Mouse Rat	Mouse - 5912 Rat - 640	C, D & E	x	x			x	x
Receptor Mechanisms in Hormone-Sensitive Cells.			Mouse Rat	Mouse - 468 Rat - 312	C & D						x
Generation and Maintenance of Transgenic, Estrogen Receptor Transgenics, and Estrogen Receptor Knockout Breeding Colonies.			Mouse	66779	C						

Appendix 6

Summary of Animal Housing and Support Sites

Redacted by agreement

Redacted by agreement

National Institutes of Health

Redacted by agreement

National Institutes of Health

Redacted by agreement

National Institutes of Health

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

INITIAL NIEHS MEDICAL EXAMINATION INSTRUCTIONS

In order to assist you in scheduling your occupational health medical examination, the following materials are included with this package:

1. Comprehensive Health History
2. Occupational History
3. Occupational Record
4. NIEHS Allergy Questionnaire

To schedule your examination, please complete all forms and questionnaires in this package and return them to:

Health Unit Nurse

Redacted by agreement

You will be called by the Health Unit Nurse who will schedule the examination. All examinations are given in the Health Unit. Please feel free to contact the Health Unit if you have any questions.

NIEHS
Occupational Health Program

National Institute of Environmental Health Sciences, U.S. Public Health Service, HHS.
Research Triangle Park, NC

COMPREHENSIVE HEALTH HISTORY

GENERAL INFORMATION

Name: _____ SS #: _____ Date: _____
Last, First Initial

Address: _____ Home phone: _____ Marital Status:

_____ Work phone: _____ ☐ Single ☐ Married ☐ Separated ☐ Divorced ☐ Widow/er

Place of birth: _____

Birthdate: _____ Age: _____ Sex: _____ Race: _____

Hobbies, interests: _____

Personal physician: _____

Physician address: _____

Purpose of Exam: _____

Name, address and phone number of person to notify in case of emergency:

_____

_____

PERSONAL HEALTH HISTORY

1. Do you have any medical problems?
   ☐ No ☐ Yes (please describe): _____
2. Have you ever been hospitalized or had any operations?
   ☐ No ☐ Yes (please describe): _____
3. Have you had serious illnesses or injuries not described above?
   ☐ No ☐ Yes (please describe): _____
4. Do you have any allergies?
   ☐ No ☐ Yes (please list): _____
5. Do you take medicines of any kind? Please note dose, size and frequency, if known.
   ☐ No ☐ Yes (please list): _____
6. When was your last tetanus shot? _____
7. What do you and your partner use to prevent pregnancy? _____
8. FOR WOMEN: Number of pregnancies? _____ Number of miscarriages or abortions _____
   Date of last period _____ Number of living children _____

Adapted with permission from Duke Occupational Health Services (5/92)

9. Have you ever had any of the following:	Yes	No
Allergic reactions to medicines or injections		
Dermatitis or skin problems		
Defective vision		
Worn glasses or contact lenses		
Glaucoma		
Cataracts		
Hearing loss		
Worn a hearing aid		
Ruptured ear drum		
Thyroid problems		
Hay fever or respiratory allergy		
Asthma		
Bronchitis		
Emphysema		
Collapsed lung		
Rheumatic fever		
Heart murmur		
Angina		
Heart attack		
Other heart problem		
High blood pressure		
Stomach or duodenal ulcer		
Gallstones or gallbladder problems		
Hepatitis/jaundice/liver problems		
Kidney stones		
Kidney, bladder or prostate infection		
Hernia		
Arthritis or joint problems		

	Yes	No
Back problems		
Back injury		
Cancer/tumor/leukemia		
Anemia or blood problem		
A positive TB (tuberculosis) test		
Diabetes		
Stroke		
Epilepsy or seizures		
Fainting spells		
Psychological or emotional problems		
Mental illness		
Rubella		
Radiation treatment to head or neck		
Pregnancy/childbirth (self or spouse)		
Trouble getting pregnant (self or spouse)		
A child with a birth defect		
Miscarriage/stillbirth (self or spouse)		
Others (list below):		

SYMPTOMS REVIEW

Are you currently having problems with:

	Yes	No
Persistent rash or hives		
Frequent or severe headaches		
Recurrent fevers or night sweats		
Enlarged lymph nodes or persistent lumps		
Persistent eye irritation or blurred vision		
Poor balance/dizziness/vertigo		
Ringing in the ears		
Persistent nose irritation or poor sense of smell		
Teeth or gums		
Persistent hoarseness or difficulty swallowing		
Frequent cough/coughing up phlegm or blood		
Shortness of breath with exertion		
Wheezing		
Sensation of smothering		
Chest pain or tightness with exertion		
Palpitations/irregular heartbeat		
Stomach pain		
Recurrent nausea or vomiting		
Blood in the stool/black, tarry stools		
Recurrent diarrhea or constipation		

	Yes	No
Difficult or painful urination		
Blood in the urine		
Protein in the urine		
Impotence/loss of libido/other problems with sex		
Changing mole or sore that does not heal		
Unexplained weight change greater than 10 pounds		
Recurrent joint or back pain		
Persistent pain/numbness/tingling in hands or feet		
Weakness in arms or legs		
Tremor of hands		
Memory problems/forgetfulness/poor concentration		
Easy fatigability (get tired quickly)		
Trouble sleeping/insomnia/nightmares		
Stress affecting home life, work or sleep		
Fear of tight or enclosed spaces		
Unusual bouts of anger or irritability		
Depression		
Psychological or emotional problems or concerns		
Women: Irregular or painful menstrual periods		
Breast pain, lumps or discharge		

FAMILY HISTORY

Has any relative (grandparents, parents, brother or sister) in your family had any of the following problems?

	Yes	No	Relationship to you
High blood pressure			
Heart attack/heart disease at an early age			
Diabetes ("Sugar")			
Tuberculosis			
Asthma or allergies			
Thyroid problems			
Sickle cell anemia or trait			
Stroke			
Psychological problems or treatments			
Mental illness			
Cancer or leukemia			List Type:
Do any other diseases run in you family?			List:

HEALTH HABITS

1. *Do you smoke cigarettes?* ☐No ☐Yes

   If yes, how many packs per day?_____ How many years have you smoked? _____

   If no, did you smoke in the past? ☐No ☐Yes

   How many packs per day did you smoke? _____ How many years did you smoke?_____

   When did you quit?_____

2. On the average, how much alcohol do you drink each week?

   beer _____ cans wine _____ glasses liquor _____ drinks

3. Do you wear seat belts? ☐never or rarely ☐sometimes ☐almost always

4. Do you get regular strenuous exercise outside of your job?

   ☐never or rarely ☐1-2 times/week ☐3 or more times/week

   What kind of exercise? _____

5. Do you see a dentist regularly? ☐No ☐Yes

6. Do you have your blood pressure checked regularly? ☐No ☐Yes

7. Do you examine your skin? ☐No ☐Yes

8. When was your last stool test for hidden blood? _____

9. When was your last sigmoid or proctoscope test? _____

10. For women: Do you have Pap smears on a regular basis? ☐No ☐Yes How often? _____

    Date of last Pap smear (mo/year): _____

    Do you do breast self-examinations each month? ☐No ☐Yes

11. For men: Do you examine your testicles each month? ☐No ☐Yes

Doctor's Notes:

Impression, summary and signature: _____

Check, if continued on progress note ☐

Company or institution name: City, State:	CHEMICAL EXPOSURES (List substances you used or worked around daily or frequently including dusts, fumes, chemicals and organic substances):
Dates of service: ☐ Full time ☐ Part time From (mo/yr): To (mo/yr):	
Classification or Title:	OTHER HAZARDS (List physical or other stresses, e.g. noise, to which you were exposed daily or frequently):
What work did you do? (describe):	PROTECTIVE EQUIPMENT Worn (Such as gloves, respirator, protective clothing, etc.):

Company or institution name: City, State:	CHEMICAL EXPOSURES (List substances you used or worked around daily or frequently including dusts, fumes, chemicals and organic substances):
Dates of service: ☐ Full time ☐ Part time From (mo/yr): To (mo/yr):	
Classification or Title:	OTHER HAZARDS (List physical or other stresses, e.g. noise, to which you were exposed daily or frequently):
What work did you do? (describe):	PROTECTIVE EQUIPMENT Worn (Such as gloves, respirator, protective clothing, etc.):

Company or institution name: City, State:	CHEMICAL EXPOSURES (List substances you used or worked around daily or frequently including dusts, fumes, chemicals and organic substances):
Dates of service: ☐ Full time ☐ Part time From (mo/yr): To (mo/yr):	
Classification or Title:	OTHER HAZARDS (List physical or other stresses, e.g. noise, to which you were exposed daily or frequently):
What work did you do? (describe):	PROTECTIVE EQUIPMENT Worn (Such as gloves, respirator, protective clothing, etc.):

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OCCUPATIONAL HISTORY

Name: SS#: Date:

Please start with your most recent job and list every job classification or title you have held. This job history is essential background information which is needed to make an accurate interpretation of your health testing results. Please give as much information as you can.

Company or institution name: City, State: Dates of service: ☐Full time ☐Part time From (mo/yr): To (mo/yr):	CHEMICAL EXPOSURES (List substances you used or worked around daily or frequently including dusts, fumes, chemicals and organic substances):
Classification or Title:	OTHER HAZARDS (List physical or other stresses, e.g. noise, to which you were exposed daily or frequently):
What work did you do? (describe):	PROTECTIVE EQUIPMENT Worn (Such as gloves, respirator, protective clothing, etc.):

Company or institution name: City, State: Dates of service: ☐Full time ☐Part time From (mo/yr): To (mo/yr):	CHEMICAL EXPOSURES (List substances you used or worked around daily or frequently including dusts, fumes, chemicals and organic substances):
Classification or Title:	OTHER HAZARDS (List physical or other stresses, e.g. noise, to which you were exposed daily or frequently):
What work did you do? (describe):	PROTECTIVE EQUIPMENT Worn (Such as gloves, respirator, protective clothing, etc.):

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OCCUPATIONAL RECORD

Name: SS#:

*Address (*if new): *Phone: ( )

INITIAL VISIT	SUBSEQUENT VISITS			
Division:	Job changes:	Job changes:	Job changes:	Job changes:
Lab. Branch:				
Building/Rm. No.:				
Phone Extension:				
Supervisor:				
Job title:				
Description of job activities:				
DATE:				

INITIAL VISIT

List as completely as possible the materials, chemicals or substances with which you work:

ABOVE: Note any changes in your job activities since your last visit.

BELOW: Check whether you still work with chemicals listed at left. Add to the bottom of the list any new substances used since your last visit.

	Date started	Yes	No	Yes	No	Yes	No	Yes	No
Solvents (e.g. Benzene, Toluene, etc.)									
Carcinogens (e.g. TCDD, BaP, etc.)									

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INITIAL VISIT		SUBSEQUENT VISITS							
DATE:		DATE:		DATE:		DATE:		DATE:	
	Date started	Yes	No	Yes	No	Yes	No	Yes	No
Liquid Scintillation Cocktails									
Radioisotopes (e.g. I^{125}, P^{32}, H^3,etc.)									
Hazardous wastes									
Do you work with lab animals? (list species)									
Do you work with human blood, blood products, or human tissue? (please list)									

INITIAL VISIT		SUBSEQUENT VISITS							
DATE:		DATE:		DATE:		DATE:		DATE:	
	Date started	Yes	No	Yes	No	Yes	No	Yes	No
Other biological agents, e.g. recombinant DNA, infectious agents (list agents)									
Microwave emissions and/or LASERS									
Other hazardous materials (list)									

INITIAL VISIT			SUBSEQUENT VISITS							
DATE:			DATE:		DATE:		DATE:		DATE:	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Do you have more than one job?										
Do your work activities require heavy force, strong vibration or frequent repetition of hands, wrists or arms?										
Have you ever had difficulty wearing a respirator?										
Do you have any hobbies (such as painting, gardening, welding, woodworking, hairdressing, or scuba diving) which involve exposure to chemicals or physical hazards?										
Are there any substances in or around your home that might be harmful? (fumes, gases, insulation, pesticides, paints, or others)										
Do you live near a plant, factory, dump site, or other potential source of pollution?										
Do your home or hobby activities include heavy lifting or carrying, repetitive bending or reaching?										
On the average, how many hours per week do you work?										

Have you ever filed a compensation claim or received benefits for an occupational accident, injury or illness?										
Have you ever experienced any occupational illnesses or injuries since being employed at NIEHS? (if so, describe below)										
Have you ever been disabled or restricted for medical reasons?										
Have you ever changed jobs for health or safety reasons?										
Are there any unprotected hazards on your job?										
If so, is the Health and Safety Office aware of these problems?										
Use this space to further explain any of the answers above?										

NIEHS ALLERGY QUESTIONNAIRE 1

INTAKE MEDICAL SURVEILLANCE FORM

Please fill in all blanks or circle one choice for all questions

Part 1

1. Name: _____ _____ _____
   last first middle initial

2. Birthdate: ____ ____ ____ 3. Age: _____ 4. Sex: M F
   mo day yr (circle one)

5. Today's date: _____ _____ _____
   mo day yr

6. Race: (circle one) white black other _____

7. Current job title:_____
   Where do you work? (specific location)
   building_____ lab or branch _____
   room _____

   Date you began this job (mo)____ (yr) ____
   Date you began with this organization (mo)____ (yr) ____

8. In your current job do you handle animals or their tissue, body fluids, or cages?
   yes no

   Do you work in the animal room at least once a week?
   yes no

   If no to <u>both</u> of the above skip to question #15

9. Do you work with laboratory animals or their cages regularly (at least once a week throughout the year)? yes no

   a. <u>If yes:</u>
      1. How many days per week do you work with lab animals or their cages?
      (circle one) 1 2 3 4 5 6 7 8 more

      2. <u>During these days,</u> how many hours per day (on the average) do you work with lab animals or their cages? (circle one)
      1 2 3 4 5 6 7 8 more

   b. <u>If no:</u>
      1. Over the past 24 weeks (about six months) during how many weeks have you had lab animal contact?_____

      2. <u>During these weeks,</u> how many days per week have you worked with lab animals? 1 2 3 4 5

Intake Medical Surveillance **2**

3. On these days, how many hours per day have you worked with lab animals? 1 2 3 4 5 6 7 8

10. How many hours per week do you usually have contact with the following species?

(please circle one choice for each item)

	hours per week			
guinea pig	0	1-6	7-20	21-40
hamster	0	1-6	7-20	21-40
mouse	0	1-6	7-20	21-40
rat	0	1-6	7-20	21-40
rabbit	0	1-6	7-20	21-40
other _____ (specify)	0	1-6	7-20	21-40

11. How many hours per week are you usually involved in the following activities?

(circle one choice for each listing):

	hours per week			
handle dirty cages	0	1-6	7-20	21 or more
return clean cages	0	1-6	7-20	21 or more
receiving animals	0	1-6	7-20	21 or more
breeding room	0	1-6	7-20	21 or more
holding room	0	1-6	7 -20	21 or more
gavage or other dosing	0	1-6	7-20	21 or more
weighing	0	1-6	7-20	21 or more
sacrifice/necropsy	0	1-6	7-20	21 or more
isolators	0	1-6	7-20	21 or more
change bedding	0	1-6	7-20	21 or more
other animal room housekeeping	0	1-6	7-20	21 or more
isolated organ or tissue experiments	0	1-6	7-20	21 or more
Using animals or tissues/fluids outside animal facility	0	1-6	7-20	21or more

Intake Medical Surveillance **3**

12. When working with lab animals or their cages how often do you do the following?
(circle one choice for each item):

wear gloves		
none	**less than half the time**	**most of the time**
wear a mask		
none	**less than half the time**	**most of the time**
wear a gown		
none	**less than half the time**	**most of the time**
wear shoe covers		
none	**less than half the time**	**most of the time**
wash hands after handling animals		
none	**less than half the time**	**most of the time**

13. In all your previous jobs before this job, how many years have you been involved in handling laboratory animals?_____

14. Do you have sneezing spells, running or stuffy nose, watery or itchy eyes, coughing, wheezing or shortness of breath from working with laboratory animals or their cages?

	yes	**no**

If no, then skip to #15
If yes to the above symptoms:

a. Which of the following species cause any of these problems?
(answer for each item)

guinea pig	**yes**	**no**
hamster	**yes**	**no**
mouse	**yes**	**no**
rat	**yes**	**no**
rabbit	**yes**	**no**
other_____	**yes**	**no**
bedding only	**yes**	**no**

b. How soon after exposure to lab animals do these symptoms start? **(circle one)**

less than 10 minutes	**1**
10 minutes to 1 hour	**2**
1to 8 hours	**3**
more than 8 hours	**4**
How long do they last?	
less than 10 minutes	**1**
10 minutes to 1 hour	**2**
1 to 8 hours	**3**
more than 8 hours	**4**

c. Which symptoms do you get from working with lab animals?
(circle yes or no for each)

sneezing spells	**yes**	**no**
runny nose	**yes**	**no**
watery or itchy eyes	**yes**	**no**
coughing spells	**yes**	**no**
wheezing	**yes**	**no**
shortness of breath	**yes**	**no**
skin rashes or hives	**yes**	**no**

d. Do you take any medicines for these symptoms? **yes** **no**

15. Are there any lab animals with which you cannot work because of allergic problems?

yes **no**

If yes, which animal species? _____

16. Have you ever changed jobs or working habits because of symptoms from handling animals?

yes **no**

17. Aside from your own work, are lab animals used by others in the same room where you work?

yes **no**

Agent	Hazardous Agents type	toxicant, toxin, irritant, carcinogen
a) Biological Agents		
Aspergillus fumigatus	Biological agent - fungus	ABSL 2
Bordetella pseudohinzii	Biological agent - bacteria	ABSL 2
Burkholderia cepacia	Biological agent - bacteria	ABSL 2
Burkholderia thailandensis	Biological agent - bacteria	ABSL 2
Chromobacterium violaceum	Biological agent - bacteria	ABSL 2
E. coli (strains O111 and O18)	Biological agent - bacteria	ABSL 2
Francisella philomiragia	Biological agent - bacteria	ABSL 2
Klebsiella pneumoniae	Biological agent - bacteria	ABSL 2
Klebsiella oxytoca	Biological agent - bacteria	ABSL 1
Bordetella pertussis	Biological agent - bacteria	ABSL 2
Pseudomonas aeruginosa	Biological agent - bacteria	ABSL 2
Salmonella enterica (Ses)	Biological agent - bacteria	ABSL 2
Streptococcus pneumoniae	Biological agent - bacteria	ABSL 2
H1 hES cells	Biological Agent - human cells	ABSL 2
human carcinoma cells	Biological Agent - human cells	ABSL 2
iPS human cells	Biological Agent - human cells	ABSL 2
Influenza (A/BANGKOK,	Biological agent - virus	ABSL 2
Influenza H1N1(PR8)	Biological agent - virus	ABSL 2
Influenza A virus (Hong Kong/8/68)	Biological agent - virus	ABSL 2
Rabies helper virus	Biological agent - virus	ABSL 2
Rabies virus	Biological agent - virus	ABSL 2
recombinant Adeno-associated Virus	Biological agent - virus	ABSL 2
recombinant Adenovirus	Biological agent - virus	ABSL 2
recombinant Lentivirus	Biological agent - virus	ABSL 2+
Respiratory syncytial virus-A2 virus	Biological agent - virus	ABSL 2
Zika Virus	Biological agent - virus	ABSL 2
b) Chemical Agents		
2ME2 (2-Methoxyestradiol)	Chemical Agent	Carcinogen (Cat 1B)
6-OHDA	Chemical Agent	Irritant
Anisomycin	Chemical Agent	Toxicant
AOM (Azoxymethane)	Chemical Agent	Carcinogen (Cat 1B)
Apomorhpine	Chemical Agent	Irritant
Arsenic (Sodium Arsenite)	Chemical Agent	Carcinogen
As2O3 (Arsenic (III) oxide)	Chemical Agent	Carcinogen (Cat 1A)
AZT (3'-Azido-3'-deoxythymidine)	Chemical Agent	Antineoplastic agent
BDE-47 (Tetrabromodiphenyl Ether)	Chemical Agent	Toxicant
BHT (Butylated hydroxytoluene)	Chemical Agent	Irritant
Bleomycin sulfate	Chemical Agent	Irritant
BPA (Bisphenol A)	Chemical Agent	Irritant
BPAF (Bisphenol AF)	Chemical Agent	Irritant
BPS	Chemical Agent	Irritant
BrdU (Bromodeoxyuridine)	Chemical Agent	Antineoplastic agent
Busulfan	Chemical Agent	Antineoplastic agent
Cadmium Chloride	Chemical Agent	Carcinogen (Cat 1B), Toxicant (Cat 1B)
CAPE (caffeic acid phenethyl ester)	Chemical Agent	Potential Toxicant
Carboxylic Thromboxane A2	Chemical Agent	Antagonist
CAY10404	Chemical Agent	Inhibitor
CAY10535	Chemical Agent	Antagonist

CCl4 (Carbon Tetrachloride)	Chemical Agent	Carcinogen
Cholchicine	Chemical Agent	Carcinogen (Cat 1B), toxicant
Cigarette Smoke	Chemical Agent	Carcinogen, Irritant
Cyclosporine A.	Chemical Agent	Carcinogen (Cat 1B), Toxicant (Cat 1B)
D-amphetamine	Chemical Agent	Toxicant
DBP (Di-n-butyl phthalate)	Chemical Agent	Carcinogen, Toxicant, Irritant
DCA (dichloroacetate)	Chemical Agent	Carcinogen (Cat 2), Irritant (Cat 2)
Degarelix Citrate Salt (Firmagon)	Chemical Agent	Toxicant (Cat 1A)
DEHP (Bis(2-ethylhexyl)phthalate)	Chemical Agent	Toxin
DEN (diethylnitrosamine)	Chemical Agent	Carcinogen
DES (Diethylstilbestrol)	Chemical Agent	Carcinogen
Desferal	Chemical Agent	Potential Toxicant
DHT (Dihydrotestosterone)	Chemical Agent	Hormone
DNBS (DinitroBenzene Sulfonic acid hydrate)	Chemical Agent	Irritant
DNFB (dinitrofluorobenzene)	Chemical Agent	Irritant (Cat 2)
Doxorubicin	Chemical Agent	Antineoplastic agent
DPN (Diarylpropionitrile)	Chemical Agent	Irritant (Cat 2A)
E2 (17-β estradiol)	Chemical Agent	Hormone, Irritant
EdU (5-ethynyl-2'-deoxyuridine)	Chemical Agent	Toxin
Estradiol Benzoate	Chemical Agent	Hormone, Irritant
Estradiol Valerate	Chemical Agent	Hormone, Irritant
Flutamide	Chemical Agent	Antineoplastic agent
FSH (follicle stimulating hormone)	Chemical Agent	Hormone
Genistein	Chemical Agent	Hormone
Genistin	Chemical Agent	Hormone
hexachlorophene	Chemical Agent	Toxicant
IBOP	Chemical Agent	Antagonist
ICI	Chemical Agent	Antineoplastic agent
ICI 182,780	Chemical Agent	Antineoplastic agent
ISAP	Chemical Agent	Antagonist
L-DOPS	Chemical Agent	Irritant
Phenylephrine	Chemical Agent	Irritant (Cat 1B)
Isoproterenol	Chemical Agent	Irritant (Cat 2)
Maneb	Chemical Agent	Carcinogen
MCA (3-methylcholanthrene)	Chemical Agent	Mutagen
Medroxy-Progesterone	Chemical Agent	Hormone
Methylphenidate	Chemical Agent	Carcinogen (Cat 2), Irritant (Cat 1)
Mifepristone (RU486)	Chemical Agent	Toxicant (Cat 1B)
MPA (methoxyprogesterone acetate)	Chemical Agent	Hormone
MPP (Methylpiperidinopyrazole)	Chemical Agent	Irritant
MPTP (methylphenyltetra-	Chemical agent	Neurotoxin
Multi-walled Carbon Nanotubes	Chemical Agent	Carcinogen (Cat 2), Irritant
Naphthalene	Chemical Agent	Carcinogen
Nicotine	Chemical Agent	Toxin
Paraquat	Chemical Agent	Mutagen
PEITC (Phenethyl isothiocyanate)	Chemical Agent	Irritant (Cat 1)
PNU 282987 & 120596	Chemical Agent	Potential Toxicant
PPT (propyl pyrazole triol)	Chemical Agent	Estrogen receptor
Progesterone	Chemical Agent	Hormone
Rotenone	Chemical Agent	Irritant
Scopolamine	Chemical Agent	Toxicant
Sodium aresenite	Chemical Agent	Carcingen, Toxin
SP600125	Chemical Agent	Irritant
STZ (Streptozotocin)	Chemical Agent	Antineoplastic agent

Tamoxifen	Chemical Agent	Antineoplastic agent
TBBPA (Tetrabromobisphenol A)	Chemical Agent	Carcinogen
Trimethyltin hydroxide	Chemical Agent	Toxin
Triptorelin	Chemical Agent	Toxicant (Cat 1B)
U-46619	Chemical Agent	Irritant
U-51605	Chemical Agent	Irritant
Urethane	Chemical Agent	Carcinogen
14C-labeled TBB (2-Ethylhexyl-2,3,4,5,-tetrabromobenzoate)	Chemical Agent (rad)	Radioisotope
14C-labeled TBBPA (Tetrabromobisphenol A)	Chemical Agent (rad)	Radioisotope
14C - labeled TBPH (di(2-ethylhexyl) tetrabromophthalate)	Chemical Agent (rad)	Radioisotope
^{14}C-labeled TBP	Chemical Agent (rad)	Radioisotope

c) Physical Agents

Laser - Class 3B	Physical Agent	
Laser - Class IV	Physical Agent	
UV radiation	Physical Agent	Non-ionizing radiation

Cat 1A - Known to have carcinogenic potential for humans – largely based on evidence from human studies
Cat 1B - Presumed to have carcinogenic potential for humans – largely based on animal evidence
Cat 2 - Suspected human carcinogen. Evidence from animal and/or human studies is limited

IACUC/OB Membership Roster

Name	Degree	Membership Role	Affiliation
Don Cook	PhD	Chair/Scientist	Immunity, Inflammation, and Disease Laboratory
Redacted by agreement			
Kathy Laber	DVM	Animal Program Director/Attending Veterinarian	Comparative Medicine Branch
Redacted by agreement			

Leave Blank
Proposal #: **Click here to enter text.**
Branch: **Click here to enter text.**
Approval Date: **Click here to enter text.**
Expiration Date: **Click here to enter text.**

National Institute of Environmental Health Sciences
Animal Study Proposal

Instructions for completing the NIEHS Animal Study Proposal

A. ADMINISTRATIVE DATA:

Principal Investigator: Click here to enter text.

Laboratory or Branch: Click here to enter text. Mail Drop: Click here to enter text.

Office Phone: Click here to enter text.

Project Title: Click here to enter text.

Initial Submission ☐; Renewal ☐ of Proposal # Click here to enter text.

List the names of all individuals authorized to conduct procedures involving animals under this proposal **and attach** NIEHS form "Statement of Training and Experience for Use of Experimental Animals" for each participant:
Click here to enter text.

B. ANIMAL REQUIREMENTS:

	Species:	Strain Shorthand (for cage card):	Stock/Strain Proper Nomenclature:	Vendor Catalog number:	Age/ Weight/ or Size:	Sex:	Max. # housed at one time:	Average length of time housed:	Total #:
1	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
2	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
3	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
4	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
5	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
6	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
7	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.

C. STUDY OBJECTIVES and RATIONALE FOR USE OF ANIMALS:

1) Briefly explain in **non-technical terms** the aim of the study and how the study may benefit human or animal health or advance scientific understanding of biological processes. This **non-technical** description is directed to the lay public to facilitate understanding of the Animal Study Proposal and should be understandable at an eighth-grade level.
Click here to enter text.

2) Explain your rationale for animal use.
Click here to enter text.

3) Justify the appropriateness of species.
Click here to enter text.

4) Justify the number of animals to be used.

A) ☐ Number of animals (adults and pups/embryos) required for breeding, production, and maintenance is calculated by the following breeding equation(s):
(ex. 2 pairs x 6 pups/litter x 8 litters/year x 3 years= total # of pups produced +10% safety factor + original breeders= total)
Click here to enter text.

Total number of animals needed: Click here to enter text.

B) ☐Number of animals required for experimental studies are based on statistical calculations as follows:

	Exp #1	Exp #2	Exp #3	Exp #4
Primary experimental outcome (e.g., body weight, tumor present/absent, gene expression)	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Expected magnitude of effect: (e.g., 2 gram decrease, 3-fold increase, 30% change)	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Expected standard deviation or range, if applicable:	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Statistical method(s) to be used: (e.g., ANOVA, Chi square, or Student's T-test)	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Power required: 80%, 90%, Other (define) or 95% confidence interval width	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Based on these values, the calculated number of animals required per treatment group:	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Online calculators such as StatPages are available to assist with statistical design as well as consultation with the ACUC Biostatistician.

C) ☐Other (example: amount of tissue required to perform in vitro analyses):
Click here to enter text.

D. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:
Briefly explain the experimental design and specify all animal procedures to be utilized. Include injections or inoculations (substances, i.e. infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules), blood withdrawals (volume, frequency, withdrawal sites, and methodology), non-survival surgical procedures **(Provide details of survival surgical procedures in Section E.)**, radiation (dosage

and schedule), methods of restraint (i.e. restraint chairs, vests, harnesses, slings, etc.), animal identification methods (i.e. ear punches/notches, ear tags, tattoos, collar, cage card, etc.) and other procedures (i.e. survival studies, tail biopsies, etc.). State what resultant effects, if any, the animals are expected to experience (i.e. pain or discomfort, ascites production, etc.). Experimental endpoint criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal.

1) Site of animal procedures:
☐Animal Procedures will be conducted only in the NIEHS Animal Facilities (No room #s are required)

☐Animals will be transported to an NIEHS laboratory.
Provide the room number, indicate the procedure(s) performed there (ie. euthanasia and tissue collection), how long they will be kept there, and who will be responsible for their welfare. The NIEHS Guidelines for Maintaining Animals in Laboratories must be adhered to. Animals may be maintained outside the NIEHS animal facilities for less than 24 hours.
Click here to enter text.

2) Description of animal procedures including experimental and humane endpoints:
Click here to enter text.

☐We will follow the ARAC Guideline: Endpoints in Animal Study Proposals; morbidity section . No adverse consequences expected; VMS will monitor animals and manage clinical cases in accordance with best practices.

3) Adverse Phenotypes:
☐There are no known or expected adverse phenotypes associated with any of the strains proposed in this ASP. If adverse phenotypes are observed, VMS will monitor animals and manage clinical cases in accordance with best practices

☐Adverse phenotypes are expected. Please list the strains and describe the adverse phenotype expected as well as the clinical signs you will monitor for, the monitoring strategy, as well as the endpoints for removal from study.
Click here to enter text.

☐We will follow the ARAC Guideline: Endpoints in Animal Study Proposals; morbidity section.

4) Compounds Administered to Animals:
List all compounds including vehicles that will be administered to animals in the table below.

Compound	Pharm Grade (Y/N)	Grade, Quality, or Purity (if NPG)	Dose(s)	Route	Volume	Frequency
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.

The Non-pharmaceutical grade (NPG) compounds listed below are required for:

Compound	Rationale for the use of NPG
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.

examples include: compounds are not commercially available as pharmaceutical grade, NPG compounds have been used previously and are required for scientific compatibility

☒ Consideration has been given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use.

E. SURVIVAL SURGERY: NONE ☐ MINOR ☐ MAJOR ☐

1) Identify the surgical procedure(s) to be performed:
Click here to enter text.

2) Describe the procedure(s) and aseptic techniques to be utilized in accordance with the Guide for the Care and Use of Laboratory Animals: (May attach relevant NIEHS Surgical SOP's)
Click here to enter text.

3) Who will perform surgery and what are their qualifications and/or experience?
(See attached "Statement of Training and Experience" form)
Click here to enter text.

4) Where will surgery be performed?
NIEHS Animal facility, Procedure room ☐
NIEHS Animal facility, Surgical Suite ☐

5) Describe post-operative care required, location of post-operative care, and identify the individual who will monitor the animals:
Click here to enter text.

6) Has major survival surgery been performed on any animal prior to being placed on this study (i.e. surgery by vendor)?
No☐ Yes ☐ **(If yes, please explain)**:
Click here to enter text.

7) Will more than one survival surgery be performed on an animal while on this study?
No☐ Yes ☐ (If yes, please provide scientific justification):
Click here to enter text.

F. PAIN OR DISTRESS CATEGORY:

(See ASP Instructions Attachment III for definitions and guidelines.) Check the appropriate category(ies) and indicate the approximate number of animals in each. List animals (by species) according to procedures performed only at NIEHS, *i.e.* do not include treatments performed by the vendor.

NUMBER OF ANIMALS USED EACH YEAR	Year 1	Year 2	Year 3
USDA Column C - Minimal, Transient, or No Pain or Distress	enter #	enter #	enter #

USDA Column D - Potential Pain or Distress Relieved By Appropriate Measures List of Column D Procedures (ex: Ovex): Click here to enter text.	enter #	enter #	enter #
USDA Column E - Unrelieved Pain or Distress*** List of Column E Procedures: Click here to enter text.	enter #	enter #	enter #

***** IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. PLEASE COMPLETE AND ATTACH THE NIEHS " COLUMN E" FORM. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA AND IS AVAILABLE UNDER THE FREEDOM OF INFORMATION ACT.**

G. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

For animals indicated in Section G, USDA Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s),dosage, route and frequency of administration. Describe parameters used to monitor level of anesthesia.

Click here to enter text.

H. METHOD OF EUTHANASIA:

Indicate the proposed method of euthanasia. If a chemical agent is used, specify the dosage and route of administration. If a euthanasia method is proposed that is not recommended by the AVMA Guidelines on Euthanasia (i.e. decapitation or cervical dislocation without anesthesia) provide scientific justification why such a method must be used.

Click here to enter text.

I. HAZARDOUS AGENTS:

Use of hazardous agents requires the approval of the NIEHS Health and Safety Branch. Approved Safety protocols are required for the use of recombinant DNA, potential pathogens, toxicants, or radioisotopes. **APPROVED SAFETY PROTOCOLS MUST BE ATTACHED TO THIS ASP BEFORE ANY WORK INVOLVING HAZARDOUS AGENTS MAY COMMENCE!**

NO ☐

YES ☐LIST AGENTS AND PROTOCOL NUMBERS (IF APPLICABLE)

1) Radioisotopes

Click here to enter text.

2) Biological Agents with Pathogenic Potential

Click here to enter text.

3) Hazardous Chemicals or Drugs

Click here to enter text.

4) Recombinant DNA

Click here to enter text.

J. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS (i.e. cell lines, antiserum, etc.):

NONE ☐ (check if none)

List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.

Material:	Source:	Sterile? Y	N

Click here to enter text.	Click here to enter text.	☐	☐
Click here to enter text.	Click here to enter text.	☐	☐
Click here to enter text.	Click here to enter text.	☐	☐
Click here to enter text.	Click here to enter text.	☐	☐
		Y	N
If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)		☐	☐
Have the tested materials been passed through rodents outside of the animal facility in question?		☐	☐
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?		☐	☐
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens. **If the NIEHS Quality Assurance Laboratory (QAL) has not tested this material, it will be cleared by the NIEHS QAL before use in animals.**		☐	☐

_____Initials of Principal Investigator.

K. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY:

List any special housing, equipment, or animal care requirements (i.e., special caging, environmental conditions, water, feed, or waste disposal, etc.). Controlled Substances must be obtained through the NIEHS Controlled Drug Officer and used and maintained according to NIEHS Policy on Controlled Substances.

Click here to enter text.

☐**Single housing (Provide Justification):** enter text
☐**Caging and environmental conditions:** enter text
☐**Water and feed:** enter text
☐**Controlled Substances will be obtained through the NIEHS Controlled Drug Officer and used and maintained according to NIEHS Policy on Controlled Substances.:** enter text
☐**Other:** enter text

L. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

Consult the NIEHS ASP Instructions and the CMB Office for suggestions of sources for use in documenting the certifications required below.

1. I certify that I have attended the NIEHS investigator training course. (Attach copy of NIEHS "Statement of Training and Experience for Use of Experimental Animals" form for each participant.)

2. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal and have received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns.

3. I certify that all individuals working on this proposal are participating in the NIEHS Occupational Health Surveillance Program.

4. I certify that I have determined that the research proposed herein is **not unnecessarily duplicative** of previously reported research.

5. **REQUIRED BY LAW FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section F)**: I certify that I have reviewed the pertinent scientific literature and the sources and /or databases as **noted below** and have found no valid alternative to any procedure described herein which may cause more than momentary or slight pain or distress. ***The methods and sources used in my search included the following*** (*May include consultations, experience in field*, database *searches etc.; if performed, database searches must include the database searched, date of the search, years*

covered by the search, and the keywords and/or search strategy used - ***The critical issue is to convey that alternatives were seriously considered): (May attach NIEHS Alternative Consideration Form)***

Click here to enter text.

6. I will inform the ACUC of any proposed significant changes in this study.

Principal Investigator
Name:Click here to enter text.

Signature_____ Date:_____

M. CONCURRENCES:

Laboratory/Branch Chief/Scientific Director certification of review and approval on the basis of scientific merit. (Signature of next higher level of review required for proposals submitted by a Laboratory or Branch Chief.)
Name: Click here to enter text.

Signature: _____Date: _____

Animal Program Director certification of review of animal procedures and appropriate use of analgesics, anesthetics and tranquilizers.

Name: Kathy Laber, DVM

Signature: _____Date_____

N. FINAL APPROVAL:

Certification of review and approval by the NIEHS Animal Care and Use Committee:

CHAIRPERSON:
Name: Don Cook, PhD.

Signature: _____Date: _____

AMENDMENT
TO NIEHS ANIMAL STUDY PROPOSAL

ASP Number, Lab Branch: enter text **Amendment #:** enter text

Principal Investigator: enter text **Using Investigator:** enter text.

Title of ASP: enter text

Amendment to the NIEHS ANIMAL STUDY PROPOSAL identified above is requested for the following reason(s):

☐ **ADD NEW PARTICIPANT(S)**

Name(s) of New Participant(s): enter text
☐ **"Statement of Training and Experience for the Use of Experimental Animals" is attached**
☐ **Participant has enrolled in the NIEHS Occupational Health Monitoring Program.**

☐ **CHANGE SPECIES OR ADD MORE ANIMALS**

	Species: (Ex. Mouse)	**Strain Shorthand:** (31 character limit for cage card):	**Stock/Strain Proper Nomenclature:**	**Vendor/ Catalog number:**	**Age/ Weight/ or Size:**	**Sex:** (M or F)	**Max. # housed at one time:**	**Average length of time housed:**	**Total #:**
1									
2									
3									
4									
5									
6									

Justify the appropriateness of species:

Justify the number of animals to be used

☐ Breeding is based on the following calculations (describe):

☐ or experiments, sample size justification is as follows (consult with Dr. Grace Kissling, Biostatistician) Describe:

☐ Other (e.g., tissue harvest) Describe:

Adverse Phenotypes

This section is NOT intended for the description of adverse consequences as a result of the experimental procedures. Adverse consequences should be described under "CHANGES IN ANIMAL PROCEDURES OR SURGICAL PROCEDURES".

☐ There are no known or expected adverse phenotypes associated with any of strains proposed in this amendment. If adverse phenotypes are observed, VMS will monitor animals and manage clinical cases in accordance with best practices

☐ Adverse phenotypes are expected. Please list the strains and describe the adverse phenotype expected as well as the clinical signs you will monitor for, the monitoring strategy, as well as the endpoints for removal from study (describe):

☐ **We will follow the ARAC Guideline:** Endpoints in Animal Study Proposals; morbidity section

PAIN OR DISTRESS CATEGORY:
Indicate the number of animals in each category. List animals (by species) according to procedures performed only at NIEHS, *i.e.* do not include treatments performed by the vendor.

	Total #:
USDA Column C - Minimal, Transient, or No Pain or Distress	
USDA Column D - Potential Pain or Distress Relieved By Appropriate Measures List of Column D Procedures (ex: Ovex): enter text.	
USDA Column E - Unrelieved Pain or Distress List of Column E Procedures: enter text.	

***All Col. D and E procedures require an alternative consideration** (Library instructions)

☐ An Alternative Consideration Form is attached; OR ☐ already on file
☐ A Justification for Col. E Procedures is attached; OR ☐ already on file

☐ **PROCEDURES ARE AS DESCRIBED IN THE ASP (no new procedures are proposed)**

☐ **CHANGES IN ANIMAL PROCEDURES OR SURGICAL PROCEDURES (brief sequential description)**:

☐ **We will follow the ARAC Guideline:** Endpoints in Animal Study Proposals; morbidity section.
No expected adverse consequences expected; VMS will monitor animals and manage clinical cases in accordance with best practices

Compounds/Agents Administered to the Animals in this Amendment:

Compound	**Haz. Agent (Y/N)**	**Safety Protocol #**	**Pharm Grade (Y/N)**	**Grade, Quality, or Purity (if NPG)**	**Dose(s)**	**Route**	**Volume**	**Frequency**
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.
enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.	enter text.

The Non-pharmaceutical grade (NPG) compounds listed below are required for:

Compound	**Rationale for the use of NPG**
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.
Click here to enter text.	Click here to enter text.

examples include: compounds are not commercially available as pharmaceutical grade, NPG compounds have been used previously and are required for scientific compatibility

☐ Consideration has been given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use.

☐ are biological material or animal products (i.e. cell lines, antiserum, etc.) which may need to be screened by the QAL prior to inoculation. Please list:

☐ **SPECIAL ANIMAL CARE CONCERNS OR ENVIRONMENTAL REQUIREMENTS (describe):**

☐ **Single housing** (Provide Justification):
☐ **Caging and environmental conditions** (describe):
☐ **Water and feed** (describe):
☐ **Controlled Substance (will be obtained through the NIEHS Controlled Drug Officer and used and maintained according to NIEHS Policy on Controlled Substances:**
☐ **Other** (describe):

☐ **OTHER CHANGES (Describe):**

Signatures:

Principal Investigator
Name: enter text

Signature: _____(Date)

Lab/Branch Chief/Scientific Director (The signature of the SD is required for proposals submitted by a Laboratory or Branch Chief.)
Name: enter text

Signature: _____(Date)

Animal Program Director
Kathy Laber, DVM

Signature :_____(Date)

Chairperson, ACUC
Don Cook, PhD

Signature: _____(Date)

NIEHS 07/2017

AMENDMENT
ADD NEW PARTICIPANTS
TO AN NIEHS ANIMAL STUDY PROPOSAL

ASP Number, Lab Branch: enter text **Amendment #:** enter text.

Principal Investigator / Using Investigator: enter text

Title of ASP: enter text

NEW PARTICIPANT(S): list name(s)

☐ **"Statement of Training and Experience for the Use of Experimental Animals" is attached**

☐ **Participant has enrolled in the NIEHS Occupational Health Monitoring Program.**

Signatures:

Principal Investigator

Name: enter text

Signature: _____(Date)

Lab/Branch Chief/Scientific Director (The signature of the SD is required for proposals submitted by a Laboratory or Branch Chief.)

Name: enter text

Signature: _____(Date)

Animal Program Director

Kathy Laber, DVM

Signature :_____(Date)

Chairperson, ACUC

Don Cook, PhD

Signature: _____(Date)

NIEHS 01/2016

Appendix 19- Annual Review Form

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709
Website: www.niehs.nih.gov

Date: ###, 2015

From: NIEHS Animal Care and Use Committee

Subject: **Annual Review of Animal Use Activities**

To: Principal Investigator: **Dr. ###**

Project Number/Branch: **####-####, ###**

Title of Project:

Due Date: **###, 2015**

NIEHS Animal Study Proposals are approved for a maximum of three years. However, the Animal Welfare Act Regulations require a review of all activities involving animals at least annually. The NIEHS Animal Care and Use Committee requests that the attached annual review checklist be completed as a self-evaluation of the research project for which you are listed as the PI. Please complete this form and return it to Susan Schnurr (ext. 1-1960), ACUC Coordinator, at Mail Drop D1-03/ Rm D106 by
March 18, 2015.

1.	I wish to inactivate this study. No further work will be conducted under this ASP and **no animals are currently housed under this ASP**. (If you choose to inactivate the study, please mark the box in the right column, sign the form and return it to MD: C1-05 without completing the review)	☐

REVIEW

	Proposal and Personnel	
2.	Do the PI and all listed participants have access to the most recent version of the approved ASP and any amendments?	☐Yes ☐ No
3.	Are all personnel who handle animals listed as participants on this ASP?	☐Yes ☐ No
4.	Are all participants on the ASP appropriately trained (participants are required to attend the Humane Care and Use of Animals in Research Seminar and each person must also complete a web-based refresher course on a triennial basis.)	☐Yes ☐ No
5.	Is each room, outside the animal facility, where animals are taken, listed on the ASP?	☐Yes ☐ No ☐ N/A
	Study Procedures	
6.	Are the procedures currently being used the same as those described in the ASP and associated amendments?	☐Yes ☐ No
7.	Are the species, strain, and numbers of animals currently being used consistent with those in the approved ASP?	☐Yes ☐ No
8.	Have amendments been submitted for any changes in procedure?	☐Yes ☐ No
9.	Are the scientific and humane endpoints still the same as the ones outlined in the original ASP and associated amendments?	☐Yes ☐ No
10.	Do the USDA Pain and Distress Categories in the ASP accurately reflect what has been observed thus far in the study? If you indicate "No", the study should be	☐Yes ☐ No

	amended to account for any unexpected effects from the procedures used in the past year and the Pain/Distress Category should be modified.	
	Anesthesia/Analgesia	
11.	Are the methods of anesthesia the same as what is described in the ASP?	☐Yes ☐ No ☐ N/A
12.	Are anesthetized animals being monitored as described in the ASP?	☐Yes ☐ No ☐ N/A
13.	Are analgesic/anesthetic dosages, frequency, and routes of administration the same as originally outlined in the ASP or amendments?	☐Yes ☐ No ☐ N/A
	Rodent Survival Surgery	
14.	Are all the participants performing surgery identified in the ASP?	☐Yes ☐ No ☐ N/A
15.	Is the surgeon properly trained in anesthetic, surgical, and post-operative monitoring techniques?	☐Yes ☐ No ☐ N/A
16.	Is an aseptic technique used?	☐Yes ☐ No ☐ N/A
17.	Is the frequency of post-surgical monitoring as described in the original ASP adequate?	☐Yes ☐ No ☐ N/A
	Euthanasia	
18.	Does the method of euthanasia correspond with what is described in the ASP/amendments?	☐Yes ☐ No

	Conclusion	
19.	This project will be continued with no changes ("yes" or "N/A" should be marked for all questions)	☐
20.	This project will be continued and approval of changes is requested. (Please complete and attach an AMENDMENT TO NIEHS ANIMAL STUDY PROPOSAL form if "No" is marked for any question).	☐
21	If changes are requested at any time during the coming year, an AMENDMENT TO NIEHS ANIMAL STUDY PROPOSAL will be submitted to the ACUC.	☐ Agree

Additional Comments noted during annual review:

Signatures:
Principal Investigator
Name:

Signature: _____(Date)
Lab/Branch Chief /Scientific Director (The signature of the SD is required for annual reviews submitted by a Laboratory or Branch Chief.)
Name:

Signature: _____(Date)
Animal Program Director
Kathy Laber, DVM

Signature: _____(Date)
Chairperson, ACUC
John D. Roberts, PhD

Signature: _____(Date)

MINUTES OF MEETING
NIEHS ANIMAL CARE AND USE COMMITTEE
May 25; 1:00 pm
Lake View Conference Room

Members Present: Dr. Don Cook (Chair), Redacted by agreement Redacted by agreement Dr. Kathy Laber, Redacted by agreement Redacted by agreement (11/13 members present; quorum assured)

Also Present: Redacted by agreement Redacted by agreement

Members Absent: Redacted by agreement

The minutes of the April, 27, 2017 ACUC meeting were approved.

Health and Safety Branch personnel: Redacted by agreement was introduced as the new Health and Safety Officer and Redacted by agreement were introduced as new safety hygienists.

ARAC Meeting: The May ARAC meeting was cancelled and the next meeting is scheduled for June 28, 2017.

VMS Monthly Report: Redacted by agreement reported that 2 studies were monitored by Vet Med with no MTRs submitted.

Electronic Protocol Form: Dr. Laber reported that the electronic form in eSirius is set to go live for use by investigators in late summer – starting with 3-year renewals. 2 beta tests have been performed by select investigators and entry went well but issues with the pre-review process are under investigation. The ACUC Office is hiring someone to assist with implementation.

Annual Review of Animal Use Activities for May were approved.

Distributed to ACUC
Email:
Item for discussion at the April 2017 ACUC meeting; April 10, 2017 Redacted by agreement

Distributed to NIEHS ASP Participants
Email:

Each of the following proposals and amendments was reviewed for all issues covered in the instructions to investigators including rationale for work, appropriateness of animal model and animal numbers used, method of euthanasia, surgical and post-operative care and issues related to pain and distress of animals. All actions, unless noted otherwise, reflect a unanimous action on the part of committee members present and no minority viewpoints were expressed during the ACUC deliberations.

NIEHS ANIMAL STUDY PROPOSALS AND AMENDMENTS – PROPOSED FOR FULL COMMITTEE REVIEW

Project Number, Branch	Principal Investigator	Project Title	ACUC Reviewer
Redacted by agreement		**(APPROVED)** Mechanisms of Fertilization; Change in Animal Procedures: Add CARD HyperOva reagent in conjunction with hCG for superovulation procedures). **(APPROVED)** Adult Neurogenesis in the Mouse Brain; (Change in Animal Procedures: Add 3 smaller doses of Zika ($5x10^3$ FFU in 10 ul) vs. 1 dose described in the ASP). **(APPROVED)** The Role of Lipids in Pulmonary Innate Immunity; (Add More Animals: Add 456 C57BL/6 mice; Change in Animal Procedures: Add Lentivirus delivery OP (followed by approved LPS and thymocyte delivery)).	Redacted by agreement
(b)(5)			
Redacted by agreement		**(APPROVED)** Investigation of Mechanisms underlying Ozone-induced Lung Injury. (**Renewal**) **(APPROVED)** Sir2 and Nuclear Receptor in Aging and Age-associated Disorders; (Add a New Participant: Add Redacted by agreement Add More Animals: Add 48 NU/J mice; Change in Animal Procedures: Add twice daily injections for STF-118804 (NAMPT inhibitor) for 20 days following cancer cell injection).	Redacted by agreement

Redacted by agreement listed as the PI on the study, left the room during deliberations and voting and did not contribute to a quorum.

(b)(5)

Redacted by agreement	**(APPROVED)** Examination of Free Radicals In Vivo and In Vitro. **(Renewal)** **(APPROVED)** Physiological Function of TAK1 Receptor. **(Renewal)** **(APPROVED)** Use of Transgenic Mice to Study Noradrenergic Neuron Development, Diversity, and Function; (Add More Animals: Add 432 Hoxb1cre Dbh:Flpo R/C:FL-hM3Dq mice; Change in Animal Procedures: Add fear conditioning behavior test). **(APPROVED)** Regulation and Function of Mineralocorticoid Receptors in Hippocampal CA2 Neurons; (Add More Animals: Add 60 Am2-icre/ERT2(2) and 20 ROSA-tdTomato9(2) mice; Change in Animal Procedures: Add 2 IC injections of AAV in conjunction with Tamoxifen dosing IP; Add 3 new AAV viruses). **(APPROVED)** The Role of Steroid Hormones in Gastric Function; (Change in Animal Procedures: Add adrenalectomies performed in-house followed by or in conjunction with approved DHT pellet implant). **(APPROVED)** The Role of Steroid Hormones in Gastric Function; (Add More Animals: Add 106 C57BL/6 mice; Change in Animal Procedures: Add clodronate liposomes IP before and after in-house adrenalectomies).	Redacted by agreement

Redacted by agreement	**(APPROVED)** Repetitive Element and Mitochondrial Metabolism in Epigenetic Control; (Add New Participants: Add Redacted by agreement Redacted by agreement Redacted by agreement Add More Animals: add 80 FVB/NJ and 64 C57BL/6J mice; Change in Animal Procedures: Add rotenone diet for dams prior to and just following mating; Add superovulation procedures).	Redacted by agreement
Redacted by agreement	The Interaction of Innate and Adaptive Immunity in a Murine Model of Lung Allograft Rejection. **(No Longer Active)** Creation, Characterization, and Maintenance of Conditional Knockout Mice Expressing Wildtype and Cre Deletions of EMP2 Protein. **(No Longer Active)** Recovery of Injured Bronchial Epithelium Following Exposure to Multi-walled Carbon Nanotubes in C57BL/6 Mice. **(No Longer Active)** Mouse Liver S9 Preparation. **(No Longer Active)**	

NIEHS ANIMAL STUDY PROPOSALS AND AMENDMENTS - REVIEWED BY DESIGNATED MEMBER REVIEW

Project Number, Branch	Principal Investigator	Project Title	Approval Date ACUC Reviewer
Redacted by agreement		In Vivo Study of the Role of Acetylcholine in Learning and Memory; (Add More Animals: Add 348 Floxed M1 x AChRa2-cre mice; Change in Animal Procedures: Add a new virus (AAV9.hSyn.DIO.eGFP. WPRE) and some minor procedural changes). **(Previously Not Approved)**	April 27, 2017 Redacted by agreement
		DeMayo Lung Research Protocol; (Add	April 28. 2017

Redacted by agreement	More Animals: Add 620 more mice and two new strains CCSPiCreLkb1fRLuc and CCSPiCreLkb1fCas9RLuc; Change in Animal Procedures: Add intratracheal instillations of Lentivirus and IVIS imaging procedures). **(Previously Not Approved)**	Redacted by agreement
	Roles of Brain Neuroimmune Function in Inflammation-Related Neurodegeneration; (Change in Animal Procedures: Add 24 hour fast followed by buried food behavior test).	May 23, 2017 Redacted by agreement
	Role of P450 Products in Vascular Inflammation; (Add More Animals: Add 150 C57BL/6J mice to use as controls in approved experiments).	May 10, 2017 Redacted by agreement
	Chemokine and Toll-like Receptor Adaptor Function in Pulmonary Inflammation; (Add More Animals: Add 112 Ifg8g and 112 C57BL/6 mice for approved procedures).	May 18, 2017 Redacted by agreement
	Necropsy and Tissue Collection for CMPB and NIEHS Investigators; (Add More Animals: Add 6 C3H-MTV mice for tissue collection).	May 18 2017 Redacted by agreement
	The Role of Inter-alpha-trypsin Inhibitor in Wound Healing and Angiogenesis after Injury; (Add More Animals: Add 600 SPC-cre x MyD88 (fx) [STOCK Myd88<tm1Defr> Tg(Sftpc-cre)1Blh] mice for approved procedures).	May 10, 2017 Redacted by agreement
	The Role of Inter-alpha-trypsin Inhibitor in Wound Healing and Angiogenesis after Injury; (Add More Animals: Add 20 PDGRFa -EGFP [B6.129S4-Pdgfra<tm11 (EGFP)Sor>/J] mice for tissue collection).	May 9, 2017 Redacted by agreement
	Breeding Colony for Transgenic Experimental Strains; (Add More Animals: Add Tlr5 fl/fl (Gewr), Tlr5 fl/fl (Gewr) x SPC-cre, Lyz2 Cre, Tlr5 fl/fl x Lyz2 Cre, Alb-Cre, Tlr5 fl/fl (Gewr) x Alb-Cre, Mdfx, and SPC-cre x MyD88 (fx) mouse strains and 4682 animals for colony maintenance).	May 12, 2017 Redacted by agreement

Redacted by agreement	Breeding Protocol to Produce Conditional Glucocorticoid Receptor Knockout Mice; (Add More Animals: Add LoxERa, ERa-LeKO, and ERa-Krt12KO mouse lines and 874 animals for colony maintenance).	May 22, 2017 Redacted by agreement
	Identifying the Process of Reproductive Organ Formation; (Add More Animals: Add 10 mouse lines and 3168 animals for colony maintenance).	May 22, 2017 Redacted by agreement
	Breeding and Maintenance Protocol for Studies of LC3-associated Phagocytosis as a Critical Regulator of Inflammation; (Add More Animals: Add Lyz2 Cre and CD11c-cre/Reiz mouse lines and 324 animals for colony maintenance).	May 23, 2017 Redacted by agreement
	Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders (breeding protocol); (Add a New Participant: Add Redacted by agreement Add More Animals: Add DAT-Cre and DAT-Cre/Tfam mouse lines and 327 animals for colony maintenance).	May 10, 2017 Redacted by agreement
	The Role of LC3-associated Phagocytosis in the Development of Diabetes Mellitus; (Add More Animals: Add 128 TIM mice for approved STZ procedures).	April 27, 2017 Redacted by agreement
	DeMayo Mouse Breeding Protocol; (Add More Animals: Add PRCreRosaFoxl2 and Rosa Foxl2 mouse lines and 576 animals for colony maintenance).	May 23, 2017 Redacted by agreement
	Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders; (Add More Animals: Add 11 mouse lines and 960 animals for approved procedures).	May 10, 2017 Redacted by agreement
	Generation, Initial Characterization, and Maintenance or Pgc1a SINE KO mice; (Change in Animal Procedures: Add 24 fast followed by buried food behavior test).	May 23, 2017 Redacted by agreement

Redacted by agreement	Isolation and Characterization of Stem Cells and Cancer Stem Cells; (Change in Animal Procedures: Change stock concentration of Tamoxifen from 10mg/kg to 20 mg/kg (as recommended by Jackson Lab) and a dose of 75 mg/Kg bw).	Redacted by agreement April 28, 2017
	In Vivo Study of the Role of Acetylcholine in Learning and Memory; (Add More Animals: Add 106 Pom-cre mice; Change in Animal Procedures: Add 2 AAV constructs: #479 pAAV-CAG-FLEX-tdTomato or #483 pAAV-Syn-DIO-tdTomato).	May 11, 2017 Redacted by agreement
	MBD3 Mutant Mouse Generation. **(New Proposal)**	May 18, 2017 Redacted by agreement

AMENDMENTS TO NIEHS ANIMAL STUDY PROPOSALS – MINOR ADMINISTRATIVE CHANGES

Project Number, Branch	Principal Investigator	Project Title	Approval Date
Redacted by agreement		Roles of Brain Neuroimmune Function in Inflammation-Related Neurodegeneration; (Add New Participants: Add Redacted by agreement Redacted by agreement	May 8, 2017
		Breeding Protocols: For Generating Primary Brain Neuron-Glial Cultures; (Add New Participants: Add Redacted by agreement Redacted by agreement	May 8, 2017
		Developmental Pharmacogenetics; (Add a New Participant: Add Redacted by agreement Redacted by agreement	May 10, 2017

ANNUAL REVIEW OF ANIMAL USE ACTIVITIES

Project Number, Branch	Principle Investigator	Project Title
Redacted by agreement		Receptor Mechanisms in Hormone-Sensitive Cells. **(Continue with No Changes)**

Redacted by agreement	Regulation of Nicotinic Receptor Synaptic Transmission in the Rodent Brain. **(Continue with No Changes)** Glucocorticoids Block Estrogen-Induced Uterine Growth. **(Continue with No Changes)** Chemokine and Toll-like Receptor Adaptor Function in Pulmonary Inflammation. **(Continue with Changes – See Amendment)** Study of the Function of the Nuclear ROR Receptors. **(Continue with No Changes)** The Role of Inter-alpha-trypsin Inhibitor in Wound Healing and Angiogenesis After Injury. **(Continue with Changes – See Amendments)** The Role of Inter-alpha-trypsin Inhibitor in Complement Dependent Inflammation. **(Continue with No Changes)** Receptor Mechanisms in Hormone-Sensitive Cells Derived from CRACM1 Knockout Mice. **(Continue with No Changes)** Metabolism and Disposition Studies of Brominated Components of a Commercial Flame Retardant. **(Continue with No Changes)** The Role of LC3-associated Phagocytosis in the Development of Diabetes Mellitus. **(Continue with Changes – See Amendment)** Dopamine Neurons Derived from Human ES Cells Efficiently Engraft in Animal Models of Parkinson's Disease. **(Continue with No Changes)** In Vivo Study of the Role of Acetylcholine in Learning and Memory. **(Continue with No Changes)** The Role of Tlr5 in Inflammatory and Fibrotic Disease. **(Continue with No Changes)** The Role of Steroid Hormones in Gastric Function. **(Continue with No Changes)** Differentiation and Transplantation of Pancreatic Beta-like Cells Derived from Human Embryonic Stem Cells. **(Continue with No Changes)**

SUBMISSIONS PREVIOUSLY REVIEWED AND NOT APPROVED – REQUIRING MODIFICATIONS TO SECURE APPROVAL

Project Number, Branch	Principal Investigator	Project Title	NA Date Reviewer
		(b)(5)	

MINUTES OF MEETING

NIEHS ANIMAL CARE AND USE COMMITTEE

June 22; 1:00 pm

Redacted by agreement

Members Present: Dr. Don Cook (Chair), Redacted by agreement Redacted by agreement Dr. Kathy Laber, Redacted by agreement Redacted by agreement (11/13 members present; quorum assured)

Also Present: Redacted by agreement

Redacted by agreement

Members Absent: Redacted by agreement

The minutes of the May 25, 2017 ACUC meeting were approved.

ARAC Meeting: The June ARAC meeting was cancelled and the next meeting is scheduled for July 28, 2017.

ACUC office additions: Redacted by agreement was introduced as a new employee in the ACUC office.

NIH AAALAC Site Visit: NIH had its triennial AAALAC site visit the week of June 12th. Continued Full Accreditation was recommended and further details will be discussed at the July ACUC meeting.

VMS Monthly Report: Redacted by agreement reported that no MTRs were submitted for this month's reporting period.

Contaminated Substances: Redacted by agreement presented the "Request for the use of Contaminated Substances in Research Animals at NIEHS" document. The ACUC has proposed to use the form as a mechanism for a subcommittee of the ACUC to review and either approve or deny the requested use of non-pathogenic contaminants detected during clearance procedures by the QAL. During the discussion questions were raised about how similar issues are handled at other NIH ICs and the ethical considerations of using contaminated substances. The committee felt that the ethical considerations are beyond the purview of the ACUC and that the focus of the ACUC will remain to ensure animal welfare. A subcommittee composed of Redacted by agreement will review any submissions requesting the use of contaminated substances in research animals and will approve or deny such requests. A report of the subcommittees findings will be shared with the ACUC at the following month's ACUC meeting.

Annual Review of Animal Use Activities for June were approved.

Distributed to ACUC
Email:

Distributed to NIEHS ASP Participants
Email:
Specialized animal diets- Sterilization recommendations; June 3, 2017 Redacted by agreement

Each of the following proposals and amendments was reviewed for all issues covered in the instructions to investigators including rationale for work, appropriateness of animal model and animal numbers used, method of euthanasia, surgical and post-operative care and issues related to pain and distress of animals. All actions, unless noted otherwise, reflect a unanimous action on the part of committee members present and no minority viewpoints were expressed during the ACUC deliberations.

NIEHS ANIMAL STUDY PROPOSALS AND AMENDMENTS - PROPOSED FOR FULL COMMITTEE REVIEW

Project Number, Branch	Principal Investigator	Project Title	ACUC Reviewer
(b)(5)			
Redacted by agreement		**(APPROVED)** Breeding Protocol to Produce Conditional Glucocorticoid Receptor Knock-out Mice; (Add More Animals: Add 1164 Sox2-Cre and 1164 Sox2-Cre x Nr3c1 fl/fl mice; Change in Animal Procedures: Add Tamoxifen dosing).	Redacted by agreement
(b)(5)			

Redacted by agreement	**(APPROVED)** Measurements of Neuronal Activity In Vivo; (Change in Animal Procedures: Add fear conditioning behavior test; Move 100 mice to Col. E). Redacted by agreement listed as the PI on the study, left the room during deliberations and voting and did not contribute to a quorum.	Redacted by agreement
	(APPROVED) The Role of Inter-alpha-trypsin Inhibitor in Wound Healing and Angiogenesis after Injury; (Add More Animals: Add 1200 C57BL/6 and 1200 Tlr5 mice [Col. C and D]; Change in Animal Procedures: Add antibiotic cocktail via the drinking water 1 month prior to and during approved Bleomycin exposure protocol). Redacted by agreement listed as a participant on the study, left the room during deliberations and voting and did not contribute to a quorum.	Redacted by agreement
	(APPROVED) Evaluation of Hippocampal Neural Progenitor Cells and Altered Neuroimmune Regulation as Influenced by Arsenic Exposure. **(Renewal)**	Redacted by agreement
	(APPROVED) Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders; (Change in Animal Procedures: Add IC or ICV injections of colchicine).	Redacted by agreement
	(APPROVED) DeMayo Mouse Breeding Protocol; (Add More Animals: Add 70 each of the following mouse strains [350 total, All Col. C]: Myh11CrePRflox, PRflox, Myh11Cre, Myh11CreLSLPRA, and Myh11CreLSLPRB; Change in Animal Procedures: Add non-surgical embryo transfer procedures).	Redacted by agreement
	(APPROVED) Necropsy and Tissue Collection for DNTP and NIEHS Investigators. **(Renewal)**	Redacted by agreement

Redacted by agreement	**(APPROVED)** Training and Development of Technical and Surgical Procedures, Analgesic and Anesthetic Protocols; (Change in Animal Procedures: Add an antibiotic cocktail treatment study and feces collection to study the microbiome.) Redacted by agreement listed as the PI on the study, left the room during deliberations and voting and did not contribute to a quorum.	Redacted by agreement
Redacted by agreement	Regulation of Mouse P450s by Cytochrome P450 Inducers and Inhibitors, and Selected Physiologic Manipulations. **(No Longer Active)**	
	Effects of Intranasal Exposure to Diesel Exhaust Particles with Nano Ceria in C57BL/6 Mice. **(No Longer Active)**	

NIEHS ANIMAL STUDY PROPOSALS AND AMENDMENTS - REVIEWED BY DESIGNATED MEMBER REVIEW

Project Number, Branch	Principal Investigator	Project Title	Approval Date ACUC Reviewer
Redacted by agreement		Generation and Maintenance of Developmental Pharmacogenetics Colonies; (Add a New Participant: Redacted by agreement Add More Animals: Add 108 RORasg/sg mice for breeding and tissue collection).	June 12, 2017 Redacted by agreement
		Glucocorticoid Receptor Phosphorylation Site Knock-In Mice. **(Renewal)**	June 13, 2017 Redacted by agreement
		In Vivo Study of the Role of Acetylcholine in Learning and Memory; (Add a New Participant: Redacted by agreement Redacted by agreement Add More Animals: Add 224 female Floxed M1 X AChRa2-cre mice for approved study).	June 12, 2017 Redacted by agreement

Redacted by agreement	Breeding Protocol for Studies of the Role of Cholesterol Trafficking in Lung Inflammation; (Add More Animals: Add the following mouse lines and 4674 total animal for colony maintenance: Irgm1 (flox), SPC-Cre, and SPC-Irgm1 (Fx)).	June 13, 2017 Redacted by agreement
	Functional Assessment of Hippocampal Area CA2; (Add More Animals: Add 40 Am2-GFP mice; Change in Animal Procedures: Add increased dose of DHE from 20 mg/Kg to 27 mg/Kg and 1 additional dose in conjunction with approved MECS and perfusion procedures).	June 13, 2017 Redacted by agreement
	Generation, Initial Characterization and Maintenance of P450 and Soluble Epoxide Hydrolase Transgenic Mice. **(Renewal)**	June 12, 2017 Redacted by agreement
	Breeding of Gene Targeted Mice for Experimental Analysis; (Add More Animals: Add 294 Ccl19 mice for breeding and colony maintenance).	June 7, 2017 Redacted by agreement
	Metabolism and Disposition of Studies of Brominated Components of a Commercial Flame Retardant; (Add More Animals: Add 80 SD male rats for euthanasia and tissue collection).	June 13, 2017 Redacted by agreement

AMENDMENTS TO NIEHS ANIMAL STUDY PROPOSALS – MINOR ADMINISTRATIVE CHANGES

Project Number, Branch	Principal Investigator	Project Title	Approval Date
Redacted by agreement		Roles of Brain Neuroimmune Function in Inflammation-Related Neurodegeneration; (Add a New Participant: Add Redacted by agreement	June 7, 2017

Redacted by agreement

Generation and Maintenance of Transgenic, Estrogen Receptor Transgenics and Estrogen Receptor Knockout Breeding Colonies; (Add a New Participant: Add Redacted by agreement

June 7, 2017

ANNUAL REVIEW OF ANIMAL USE ACTIVITIES

Project Number, Branch	Principal Investigator	Project Title
Redacted by agreement		Characterization of COX Mice. **(Continue with no changes)**
		Physiological Function of Glis Proteins. **(Continue with no changes)**
		Functional Assessment of Hippocampal Area CA2. **(Continue with changes – see amendment)**
		Mouse Assisted Reproductive Services – Rederivation, Cryopreservation and Embryo Transfer. **(Continue with no changes)**
		The Role of LC3-associated Phagocytosis in Autoimmune and Inflammatory Disorders. **(Continue with no changes)**
		Clinical Evaluation of LLLT, Buprenorphine, Chlorhexidine in Clinical Disease. **(Continue with no changes)**
		DeMayo Mouse Breeding Protocol. **(Continue with no changes)**
		Mechanistic Study of the Impact of Environmental Factors on Neurological Disorders. **(Continue with changes – see amendments)**
		The Role of LC3-associated Phagocytosis in Arthritis and Colitis Models. **(Continue with no changes)**

SUBMISSIONS PREVIOUSLY REVIEWED AND NOT APPROVED – REQUIRING MODIFICATIONS TO SECURE APPROVAL

Project Number, Branch	Principal Investigator	Project Title	NA Date Reviewer
(b)(5)			

SEMIANNUAL REPORT

ANIMAL CARE AND USE
PROGRAM REVIEW AND FACILITY INSPECTION
OF THE

NIEHS

APRIL 2017

Section A – Site Visits & Program Review

1) Inspections of the NIEHS animal facilities (AF), satellite holding facilities (SF), USDA-defined study areas for regulated species (SA) and areas where any surgical manipulations (Surg) are performed (as applicable) were conducted as indicated below:

Location	Type	Date	ACUC Members
Redacted by agreement	AF, Surg	2/23/2017	Cook, Redacted by agreement Laber, Redacted by agreement Redacted by agreement
	Surg	8/2/2016	Redacted by agreement
	Surg	8/19/2016	Laber, Redacted by agreement

2) Visits by at least one member of the ACUC to all remaining areas where animal activities were performed were conducted. These visits occurred during the previous six months and findings and corrective actions are described in this or the previous semiannual report.

3) Additionally, the NIEHS AAALAC program description, the Guide for the Care & Use of Laboratory Animals, 8th Edition (Guide), and the NIEHS Semiannual walk through summary was used as the basis for review of the animal care and use program.

Section B – Regulatory Compliance:

Except as noted in Sections F and G below, the facilities and program are in full compliance with the Public Health Service Policy, the Animal Welfare Act Regulations and the Guide, which were used as the basis for this evaluation.

Section C – Program Changes:

The following administrative and procedural changes have occurred since the program was last evaluated:

1) Administrative/Procedural Changes:

a) The CMB completed the transition to eSirius 3G online animal ordering in November 2016.

b) The SOP for Anesthesia, Surgery, and Post Surgery Records (with 2 fillable pdf forms – anesthesia record and surgery report) was presented to the ACUC. The SOP was modified to require a 24 hour check post-surgery. The revised SOP was approved by the ACUC in January 2017.

c) The NIEHS Animal Care and Use Committee Guideline was modified to add the amendment of single housing of mice and rats as eligible for designated member review. The guideline was reviewed and approved in January 2017.

d) The NIEHS Guidelines for Resolving Non-Compliance Issues was revised to streamline and clarify the incident review and reporting process. The SOP was approved in January 2017.

e) Rodent breeding colonies continue to expand at NIEHS. At the present time there are approximately 672 strains in the animal facility; 17 inbred mouse strains, 7 outbred mice strains, 3 F1/F2 hybrid mouse strains, 1 inbred rat strain, 5 outbred rat strains, and 638 Transgenic/Knockout/Knockin mice strains.

2) Key Personnel Changes - ACUC Chair, ACUC Attending Vet, APD, or Program Manager:

None

3) Animal Facility/Area Changes:

Facility Type (AF/SF)	**Location**	**Action** (opened, closed, under renovation, etc.)
AF	Redacted by agreement	The CAF was funded and construction began in October 2016. The facility will include a procedure room and one animal room with 3 ventilated racks and will have a restricted pathogen status that is less restricted than the current Redacted by agreement animal facilities. Completion of the ~~facility~~ is projected for ~~A~~ugust, 2017.
AF	Redacted by agreement	A biosafety cabinet has been added to accommodate additional ABSL2 studies.
AF	Redacted by agreement	The space is now available for stereotaxic surgery.
AF	Redacted by agreement	In January 2017, a new double-tiered six-foot semi-rigid isolator was installed to replace older isolators and provide space to pilot germ free mouse production and maintenance.
AF	Redacted by agreement	New LED surgery lights were installed.
AF	Redacted by agreement	A biosafety cabinet has been added to accommodate additional ABSL2 studies.
AF	Redacted by agreement	New electronic controls for the tunnel washers were installed.

Section D – Guide Departures & USDA Exceptions:

Departures from the standards of the *Guide* and exceptions to the USDA *Animal Welfare Act Regulations,* which have been approved by the Animal Care and Use Committee, include the following:

1. Departures from the Guide:

Guide Departures	***Justification (scientific, veterinary, or animal welfare)***
Rodents housed on wire flooring – Guide pg. 51	Protocols approved include a scientific justification for the use of metabolism cages if housed on perforated stainless steel flooring for greater than 24 hours. These animals are acclimated to the chambers and are monitored by the CMB Veterinary Medicine Section. No problems have been observed. Currently there are four approved studies for housing mice and rats in metabolism cages for >24 hours.
Ambient housing temperature not in accordance with Guide recommendations – Guide pg. 43	Protocols approved include a scientific justification for housing mice at 4C for a

	maximum of 16 hours in order to study specific gene expression involved in energy metabolism. These animals have been monitored by the CMB Veterinary Medicine Section and no problems have been observed.
Prolonged Restraint- Guide page 29-30	One protocol is approved for prolonged restraint for a period of three hours repeated 5 consecutive days without training to the restraint system to induce stress-related body weight. The study participants continuously monitor mice during the restraint protocol. If mice fail to acclimate within fifteen minutes as determined by intense persistent struggling behavior, the restraint protocol will be terminated.

2. Exceptions to the AWAR: There are no exceptions to the AWAR.

Section E – Previous Deficiencies & Plans:

The committee validated that the plans and schedules for deficiencies noted during the previous NIEHS program review, and facilities and laboratory inspections were achieved within the time intervals projected on the previous semiannual report.

Section F – Current Deficiencies & Plans:

Deficiencies found *over the past 6 months* during NIEHS program review, facility inspections, and laboratory inspections, are as follows:

	Deficiency	[1]M/S	Location	Correction Plan	Responsible Party	Scheduled Completion Date (mm/dd/yy)	[2]Status: C/P
1	Two bottles of controlled substances were mislabeled with incorrect expiration dates.	M	Redacted by agreement	Bottles were verified to be in date when compared to the date dispensed. The Controlled Substances Officer was counselled to verify all expiration dates at the time of distribution.	CMB	2/16/2017	C
2	Animals were singly housed. Cage sticker indicated "singly housed per protocol" but ASP was not approved for single housing.	M	Redacted by agreement	The contractor was retrained for placing stickers and verifying approval for single housing.	CMB	2/10/2017	C
3	Pre-empt was found in an animal room with no expiration	M	Redacted by agreement	The contract staff were retrained to	CMB	2/10/2017	C

	date.			reinforce the importance of dating supplies.			
4	Rat was found with injures to the face	M	Redacted by agreement	VMS was notified and an animal health report was submitted. VMS followed up with the PI and the rat was euthanized.	CMB	2/6/2017	C
5	Rats were unable to normally posture in the standard rat caging, although, based upon weight, they fell within Guide minimum space recommendations.	M	Redacted by agreement	Rats were moved into larger "double-decker" cages. The SOP has been updated to include normal posturing as a housing consideration.	CMB	2/6/2017	C
6	A 10% bleach solution was found with no expiration date.	M	Redacted by agreement	New bleach solution was placed in the room and staff were retrained to reinforce the importance of dating supplies.	CMB	2/6/2017	C
7	A fume hood certification was due for renewal.	M	Redacted by agreement	The fume hood was recertified.	CMB	2/8/17	C
8	Biohazard signage was posted without emergency contact information.	M	Redacted by agreement	The placard was updated to include emergency contact information	CMB	2/6/17	C
9	(b)(5)						

[1]M=minor; S=significant [2]C=corrected; P=pending

Section G – Reportable Events:

PHS Policy (i.e. OLAW) reportable events that occurred in the last 6 months or that are still awaiting final disposition are as follows:

SA 1st noted	Description of event	Current Status
9/2016	Mice were dosed with 5mg of Tamoxifen while the ASP is only approved for a 1mg/10g dose. Dosing resulted in morbidity and deaths. The lab was unaware that the dose was not approved	Resolved 2/2017

(b)(5)

Section H – Shared & Central Facilities:
This section does not apply to this IC.

Section I – Minority Report
There is not a minority report filed with this semiannual report.

NIEHS ACUC Member Signatures:

NIEHS ACUC Member Signatures:

Redacted by agreement

Don Cook, PhD
Chair, ACUC

Redacted by agreement

Kathy Laber, DVM

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

Redacted by agreement

(Revised - 07/2016)

NATIONAL INSTITUTES OF HEALTH
Facilities and Animal Species Inventory Table
Assurance Number: A-4149-01

IC Name: National Institute of Environmental Health Sciences

Spring Semiannual Report Submission Date: April 30, 2017

Spring Program Review Date(s):	2/23/17					

Fall Program Review Date(s):						

Bldg/Area/Rm	Facility Insp. Date(s) Spring / Fall		AF/SF; Gross Sq. Ft.	Species Housed			Average Daily Inventory		
Redacted by agreement			Redacted by agreement	1 Mice	2 Rats	3	1 42,069	2 296	3
				4	5	6	4	5	6
				1	2	3	1	2	3
				4	5	6	4	5	6
				1	2	3	1	2	3
				4	5	6	4	5	6
				1	2	3	1	2	3
				4	5	6	4	5	6
				1	2	3	1	2	3
				4	5	6	4	5	6

Member Name	Degree/Credentials	Position Title	PHS Policy Membership Role	New Member
Don Cook	PhD	Principal Investigator	Chair	☐
Kathy Laber	DVM, DACLAM	Animal Program Director	Attending Veterinarian	☐
Redacted by agreement				
				☐
				☐
				☐
				☐
				☐
				☐
				☐

Don Cook, PhD

Redacted by agreement

Attending Vet Phone #: Redacted by agreement

Date of Assessment: 7/6/2017							
ROOM No.	**USE**	**AIR SOURCE % FRESH/ RECIRCULATED**	**TREATMENT FILTERED/ ABSORBERS**	**AIR CHANGES 2017**	**RELATIVE PRESSURE To Clean Corridor**	**RELATIVE PRESSURE To Return Corridor**	**HUMIDITY CONTROL**
Redacted by agreement	Procedure	100% Fresh	HEPA Filter + Carbon Filter	21.64	N/A	-	Yes
	Animal Room	100% Fresh	85% Filter	20.12	-	+	Yes
	Animal Room	100% Fresh	85% Filter	17.26	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.30	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.62	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.71	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	20.30	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.15	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	16.83	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.50	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.99	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.30	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.32	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.32	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.32	-	+	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	27.18	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	24.30	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	32.94	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	23.47	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	HEPA Filter + Carbon Filter	20.72	-	-	Yes
	Inhalation/Procedure	100% Fresh	Rough Filter	44.47	N/A	-	Yes
	Necropsy/Procedure	100% Fresh	Rough Filter	34.95	N/A	-	Yes
	Necropsy/Procedure	100% Fresh	Rough Filter	36.55	N/A	-	Yes
	Procedure	100% Fresh	None	4.33	N/A	+	Yes
	Procedure	100% Fresh	Rough Filter	13.06	N/A	+	Yes
	Procedure	100% Fresh	Rough Filter	12.54	N/A	+	Yes
	Procedure	100% Fresh	Rough Filter	11.27	N/A	+	Yes

Redacted by agreement	Recovery	100% Fresh	Rough Filter	10.20	N/A	+	Yes
	Rodent Surgery	100% Fresh	Rough Filter	21.34	N/A	+	Yes
	Rodent Surgery	100% Fresh	HEPA Filter + Carbon Filter	16.24	N/A	-	Yes
	Rodent Imaging	100% Fresh	Rough Filter	23.12	N/A	+	Yes
	Rodent Imaging	100% Fresh	Rough Filter	20.50	N/A	+	Yes
	Procedure	100% Fresh	Rough Filter	10.91	N/A	+	Yes
	Satellite Animal Containment Facility	100% Fresh	In-Line Filter	30.33	N/A	-	Yes
	Equipment	100% Fresh	None	8.52	N/A	-	No
	Cage Wash (Soiled)	100% Fresh	None	17.13	N/A	-	Yes
	Cage Wash (Clean)	100% Fresh	Rough Filter	13.87	+	N/A	Yes
	Animal Room	100% Fresh	Rough Filter	17.62	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.24	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.30	-	+	Yes
	Isolators	100% Fresh	Rough Filter	18.39	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.29	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.19	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.09	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.16	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	15.75	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	20.01	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	15.78	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.11	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.19	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.14	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.19	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	18.31	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.97	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.03	-	+	Yes
	Animal Room	100% Fresh	Rough Filter	17.10	-	+	Yes
	Animal Room	100% Fresh	HEPA Filter	15.77	-	+	Yes
	Surgery	100% Fresh	None	3.83	N/A	+	Yes
	Necropsy/Procedure	100% Fresh	HEPA Filter	34.86	N/A	-	Yes
	Animal Room (Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	9.59	-	-	Yes
	Animal Room (Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	19.39	-	-	Yes

23- HVAC System Summary

Redacted by agreement	Animal Room (Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	23.31	-	-	Yes
	Cage Wash (Soiled)	100% Fresh	None	38.30	N/A	-	No
	Cage Wash (Clean)	100% Fresh	None	6.55	+	N/A	No
	Animal Room	100% Fresh	85% Filter	18.80	-	+	Yes
	Animal Room	100% Fresh	85% Filter	18.44	-	+	Yes
	Animal Room	100% Fresh	85% Filter	18.18	-	+	Yes
	Animal Room	100% Fresh	85% Filter	18.45	-	+	Yes
	Animal Room	100% Fresh	85% Filter	13.11	-	+	Yes
	Animal Room	100% Fresh	85% Filter	18.21	-	+	Yes
	Animal Room	100% Fresh	85% Filter	20.80	-	+	Yes
	Necropsy	100% Fresh	85% Filter	34.81	-	-	Yes
	Animal Room	100% Fresh	85% Filter	21.93	-	+	Yes
	Procedure	100% Fresh	85% Filter	18.39	N/A	-	Yes
	Surgery	100% Fresh	85% Filter	30.35	-	N/A	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	31.21	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	25.96	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	23.92	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	26.38	-	-	Yes
	Animal Room (Illinois Cubicles)	100% Fresh	85% Filter	25.45	-	-	Yes
	Animal Room (Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	11.58	-	+	Yes
	Animal Room (Current Use Non Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	12.27	-	+	Yes
	Surgery	100% Fresh	None	14.04	N/A	+	No
	Animal Room (Current Use Non Containment Rm.)	100% Fresh	HEPA Filter + Carbon Filter	11.27	-	-	Yes
	Animal Room (Current Use Non Containment)	100% Fresh	HEPA Filter + Carbon Filter	12.62	-	+	Yes
	Animal Room (Current Use Non Containment)	100% Fresh	HEPA Filter + Carbon Filter	17.14	-	+	Yes

23- HVAC System Summary

Redacted by agreement	Animal Room (Current Use Non Containment)	100% Fresh	HEPA Filter + Carbon Filter	15.27	-	+	Yes
	Animal Feed/Bedding (Clean)	95% Fresh 5% Recirc	85% Filter	21.78	N/A	+	Yes
	Animal Feed/Bedding (Dirty)	95% Fresh 5% Recirc	85% Filter	8.71	N/A	N/A	Yes
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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/Enclosure	Guiding Document Used to determine the Institution's Space Standards (Guide, Ag Guide, ETS 123, Other)	Enclosure Composition & Description**
Mouse	11.6"L x 6.5"W x 5.6"H Available floor space per vendor: 75 in.²	Less than 10 gm BW: 12 10-15 gm BW: 9 15-25 gm BW: 6 > 25 gm BW: 5 Female with litter	Guide	Polysulfone Static Microisolator
Mouse	19.0"L x 10.5"W x 6.0"H Available floor space per vendor: 142 in.²	Less than 10 gm BW: 23 10-15 gm BW: 17 15-25 gm BW: 11 > 25 gm BW: 9 Female with litter	Guide	Polycarbonate Static Microisolator
Mouse	12.71"L x 6.46"W x 7.4"H Available floor space per vendor: 82 in.²	Less than 10 gm BW: 13 10-15 gm BW: 10 15-25 gm BW: 6 > 25 gm BW: 5 Female with litter	Guide	Polysulfone 1284 style IVC

Species	Dimensions of Enclosure (cage, pen, tank*, corral, paddock, etc.)	Maximum Number Animals/Enclosure	Guiding Document Used to determine the Institution's Space Standards (Guide, Ag Guide, ETS 123, Other)	Enclosure Composition & Description**
Mouse	12.7"L x 6.42"W x 6.34"H Available floor space per vendor: 81.6 in.²	Less than 10 gm BW: 13 10-15 gm BW: 10 15-25 gm BW: 6 > 25 gm BW: 5 Female with litter	Guide	Polysulfone GM500 style IVC
Mouse	12.8"L x 6.56"W x 7.0"H Available floor space per vendor: 84 in.²	Less than 10 gm BW: 14 10-15 gm BW: 10 15-25 gm BW: 7 > 25 gm BW: 5 Female with litter	Guide	Polysulfone 1285L style IVC
Mouse	7.5"L x 3.5"W x 5.0"H Available floor space per vendor: 26 in².	Single mouse only	Other – Scientific justification per research goal limits to one mouse/cage	Acrylic (Plexiglass) TSE LabMaster - Calorimetry Measuring System
Rat	14.67L x 8.45W x 9.5H Available floor space per vendor: 124 in²	<100 gm BW: 7 100-200 gm BW: 5 200-300 gm BW: 4 300-400 gm BW: 3 400-500 gm BW: 2 >500 gm BW:1	Guide	Polysulfone IVC 1291 Style
Rat	19.0"L x 10.5"W x 8.0"H Available floor space per vendor: 134 in²	< 100 gm BW: 7 100-200 gm BW: 5 200-300 gm BW: 4 300-400 gm BW: 3 400-500 gm BW: 2 >500 gm BW: 1 Female with litter	Guide	Polycarbonate Static Microisolator

Species	Dimensions of Enclosure (cage, pen, tank*, corral, paddock, etc.)	Maximum Number Animals/Enclosure	Guiding Document Used to determine the Institution's Space Standards (Guide, Ag Guide, ETS 123, Other)	Enclosure Composition & Description**
Rat	15.51"L x 13.54"W x 8.4"H Available floor space per vendor: 140 in.^{2}	<100 gm BW: 8 100-200 gm BW: 6 200-300 gm BW: 4 300-400 gm BW: 3 400-500 gm BW: 2 >500 gm BW:2 Female with litter	Guide	Polysulfone IVC GR900 Style
Rat	18.2"L x 15.8"W x 15.9"H Available floor space per vendor: 288.6in.^{2}	<100 gm BW: 16 100-200 gm BW: 12 200-300 gm BW: 9 300-400 gm BW: 7 400-500 gm BW: 4 >500 gm BW:4	Guide	Polysulfone "Double Decker" IVCS
Rat	19.0"L x 10.5"W x 8.0"H Available floor space per vendor: 134 in. ^{2}	< 100 gm BW: 7 100-200 gm BW: 5 200-300 gm BW: 4 300-400 gm BW: 3 400-500 gm BW: 2 >500 gm BW: 1 Female with litter	Guide	Polystyrene Static Microisolator Disposable

BW = Body Weight

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

Cleaning and Disinfection of the Micro- and Macro-Environment

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

Area	Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)	Washing/ Sanitizing Frequency	Other Comments
Micro-environment			
Solid-bottom cages (static)	Girton Tunnel Washer	Minimum 1x/week	
Solid-bottom cages (IVC)	Girton Tunnel Washer	1x/week or biweekly	Once weekly, at minimum, for diabetic animals, rats. Maximum interval between cage changes is two weeks for mice. Performance standards apply.
Suspended wire-bottom or slotted floor cages	Girton Bottle Washer	Minimum 1x/week	
Cage wire bar lids	Girton Bottle Washer	Biweekly	
Filter tops	Girton Bottle Washer	Monthly	
Cage racks and shelves	Girton Rack Washer, and/or hand washing with PREempt	Monthly	Static cage shelving: Alternate monthly between rack washer and hand washing IVC racks: Every 3 months in rack washer
Cage pans under suspended cages	n/a	n/a	
Play pens, floor pens, stalls, etc.	n/a	n/a	
Corrals for primates or outdoor paddocks for livestock	n/a	n/a	
Aquatic, amphibian, and reptile tanks and enclosures	n/a	n/a	
Feeders	Girton Bottle Washer,	Daily to biweekly depending on type of feed.	Weekly or biweekly for wire bar (done at cage change); weekly for glass jars; daily for petri dishes (discarded)
Watering Devices	Girton Bottle Washer	Weekly	
Exercise devices and manipulanda used in environmental enrichment programs, etc.	All other reusable enrichment: Girton Bottle Washer	Biweekly, at minimum, with cage change.	

Transport cages	Girton Bottle Washer, disposable	Prior to use	
Operant Conditioning & Recording Chambers, Mechanical Restraint Devices (chairs, slings, etc.)	Hand Washing/Bottle Washer	Weekly/After use	• UV apparatus sanitized weekly (PREempt). • Labmaster feed and water devices sanitized at least weekly (PREempt). • Cesium irradiation animal holder sanitized after each use (MB-10 , dish detergent). • Glass metabolism cages sanitized via mechanical bottle washer after each use. • Neurobehavioral equipment sanitized by hand after each use.
Euthanasia Chambers	NA	NA	Animals euthanized in home cage.

Area	**Washing/Sanitizing Method (mechanical washer, hand washing, high-pressure sprayers, etc.)**	**Washing/ Sanitizing Frequency**	**Other Comments**
Macro-Environment			
ANIMAL ROOMS			
Floors	Squeegee, then ribbed microfiber mop with PREempt	Daily	
Walls	Ribbed microfiber mop with PREempt	Monthly	
Ceilings	Ribbed microfiber mop with PREempt	Monthly	Includes lighting fixtures
Ducts/Pipes	n/a	n/a	
Fixtures	Hand washing	Varies	Door frames/vents monthly, counters & sinks daily
CORRIDORS			
Floors	Squeegee, then ribbed microfiber mop with PREempt	Daily	
Walls	Ribbed microfiber mop with PREempt	Monthly	

Ceilings	Ribbed microfiber mop with PREempt	Monthly	Includes lighting fixtures
Ducts/Pipes	n/a	n/a	
Fixtures	Hand washing	monthly	Door frames/vents monthly

SUPPORT AREAS (Locker rooms, Restrooms, Breakrooms)			
Floors	Squeegee, then ribbed microfiber mop with PREempt	Daily	
Walls, Lockers	Ribbed microfiber mop with PREempt	Monthly	
Counter Tops, Tables, Chairs, Sinks	Hand washing	Daily	
SUPPORT AREAS (surgery, procedure, necropsy rooms)			
Floors	Squeegee, then ribbed microfiber mop with PREempt	Daily	
Walls	Ribbed microfiber Mop with PREempt	Monthly	
Ceilings	Ribbed microfiber Mop with PREempt	Monthly	Includes lighting fixtures
Counter Tops, Tables, Sinks	Hand washing	Daily	
Fixtures	Hand washing	Varies	Door frames/vents monthly, counters & sinks daily
IMPLEMENTS (note whether or not shared)			
Mops/squeegees	Not shared		Dedicated to each room, restroom, support area
Mop buckets	Not shared		Dedicated to each support area
Frog nets	n/a		n/a
OTHER			
Vehicle(s)	Hand and mechanical washing	Varies	Hand sprayed between animal transports (MB-10), then disinfected once weekly with ribbed microfiber mop (PREempt).
Other transport equipment (list)	Girton Rack washer	Varies	Carts are washed post use in rack washer.

NIEHS Bioexclusion List

MOUSE

Organisms excluded from population and import

Virus

Ectromelia virus (ECTR)
Hantavirus (HTN)
Lactate dehydrogenase (elevating) virus [LD(E)V]
Lymphocytic Choriomeningitis virus (LCMV)
Minute Virus of Mice (MVM, MMV)
Mouse Adenovirus (FL/MAV-1; K87/MAV-2)
Mouse Cytomegalovirus (MCMV)
Mouse Hepatitis Virus (MHV)
Mouse Norovirus (MNV)
Mouse Parvovirus (MPV)
Mouse Pneumonitis virus (K)
Mouse Polio Virus (TMEV, MEV, GDVII)
Mouse Rotavirus (EDIM)
Mouse Thymic Virus (MTV, MTLV)
Pneumonia Virus of Mice (PVM)
Polyoma virus (POLY)
Reovirus (REO)
Sendai (SEND, SV)

Bacteria

Bordetella bronchiseptica
Cilia-associated respiratory (CAR) bacillus
Citrobacter rodentium
Corynebacterium kutscheri
Helicobacter spp.
Listeria monocytogenes
Mycoplasma pulmonis
Pasteurella pneumotropica
Salmonella spp.
Streptobacillus moniliformis
Clostridium perfringens δ
Clostridium piliforme δ
Mycoplasma arthriditis and neurolyticum δ

Klebsiella pneumonia (select studies)
Klebsiella oxytoca (select studies)

Corynebacterium bovis *
Pseudomonas aeruginosa*
Staphylococcus aureus*

Protozoa

Encephalitozoon cuniculi
Giardia muris
Spironucleus (Hexamita) muris

Ectoparasites

Demodex musculi
Myobia musculi
Myocoptes musculinis
Polyplax serrata
Psorergates simplex
Radfordia affinis

Endoparasites

Aspicularis tetraptera
Syphacia muris
Syphacia obvelata

Cestodes

Rodentolepsis nana
Rodentolepsis diminuta

Fungi

Pneumocystis murina*

δ - Prohibited from all strains. Direct testing (anaerobic culture, ELISA, PCR, etc.) required only if gross or histological evidence suggests presence of this organism.
* - Prohibited from immunocompromised strains only.

NIEHS Bioexclusion List

RAT

Organisms excluded from population and import

Virus

Hantavirus (HTN)
Kilham rat virus (KRV, RV)
Lymphocytic Choriomeningitis virus (LCMV)
Mouse adenovirus (FL-MAV1; K87-MAV2)
Pneumonia Virus of Mice (PVM)
Rat Coronavirus (RCV/SDA)
Rat Minute Virus (RMV)
Rat Parvovirus (RPV)
Rat Theilovirus (RTV) or TMEV-like agent
Reovirus (REO)
Sendai (SEND, SV)
Toolan's rat virus (H-1)

Bacteria

Bordetella bronchiseptica
Cilia-associated respiratory (CAR) bacillus
Corynebacterium kutscheri
Helicobacter spp.
Listeria monocytogenes
Mycoplasma pulmonis
Pasteurella pneumotropica
Salmonella spp.
Streptobacillus moniliformis
Streptococcus pneumoniae
Clostridium piliforme δ
Mycoplasma arthriditis δ

Pseudomonas aeruginosa *
Staphylococcus aureus*

Protozoa

Encephalitozoon cuniculi
Spironucleus (Hexamita) muris
Giardia muris

Ectoparasites

Radfordia ensifera
Myobia musculi
Radfordia affinis
Myocoptes musculinus
Polyplax spinulosa

Endoparasites

Syphacia muris
Syphacia obvelata
Aspicularis tetraptera
Trichosomoides crassicauda

Cestodes

Rodentolepsis nana
Rodentolepsis diminuta

Fungi

Pneumocystis carinii

δ - Prohibited from all strains. Direct testing (anaerobic culture, ELISA, PCR, etc.) required only if gross or histological evidence suggests presence of this organism.
* - Prohibited from immunocompromised strains only.