Division of Comparative Medicine

Laboratory Animal Users' Handbook

Massachusetts Institute of Technology

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Message from the Director of the Division of Comparative Medicine

To All Investigators Using Animals in Research at MIT and the Whitehead Institute for Biomedical Research (WIBR):

The use of animals in research involves a number of regulatory, practical and ethical concerns. For these reasons, the Division of Comparative Medicine (DCM) and the Committee on Animal Care (CAC) have produced this latest edition of the *DCM Laboratory Animal Users' Handbook.* It provides investigators, related staff, and students with information that is essential to understanding the important issues related to using animals in research.

The Division of Laboratory Animal Medicine was established in 1974. Since its founding, the mission of the Division is to provide the veterinary care and maintenance of animal health for all animals used in biomedical research at MIT, diagnostic laboratory services, support biomedical research funded by NIH and private industry, and post-doctoral training in biomedical research. In July of 1980, the Division was designated as a separate academic unit reporting directly to the Vice President for Research and was renamed the Division of Comparative Medicine to reflect its growing participation in research and teaching. The CAC was formed in 1985 in response to new federal regulations. DCM interacts closely with the CAC to satisfy legal mandates and federal guidelines for the care of animals used in research, to assist investigators in achieving their research goals, and to maintain the highest standards of ethical oversight for the animals used in research.

The animal care and use program encompasses sixteen different departments, laboratories and centers which conduct research using animals at MIT and the WIBR. Today, DCM continues to oversee the research, teaching and care of more than 100,000 animals on a daily basis in 190,000 gross square feet in new and renovated facilities. These facilities include animal housing, dedicated procedure space, surgical facilities, diagnostic laboratories, research laboratories, and administrative / office space for DCM and the CAC. Our animal care program is routinely evaluated by local, state and federal agencies to ensure these standards are being upheld, and we have been accredited by the AAALAC international, since 1972.

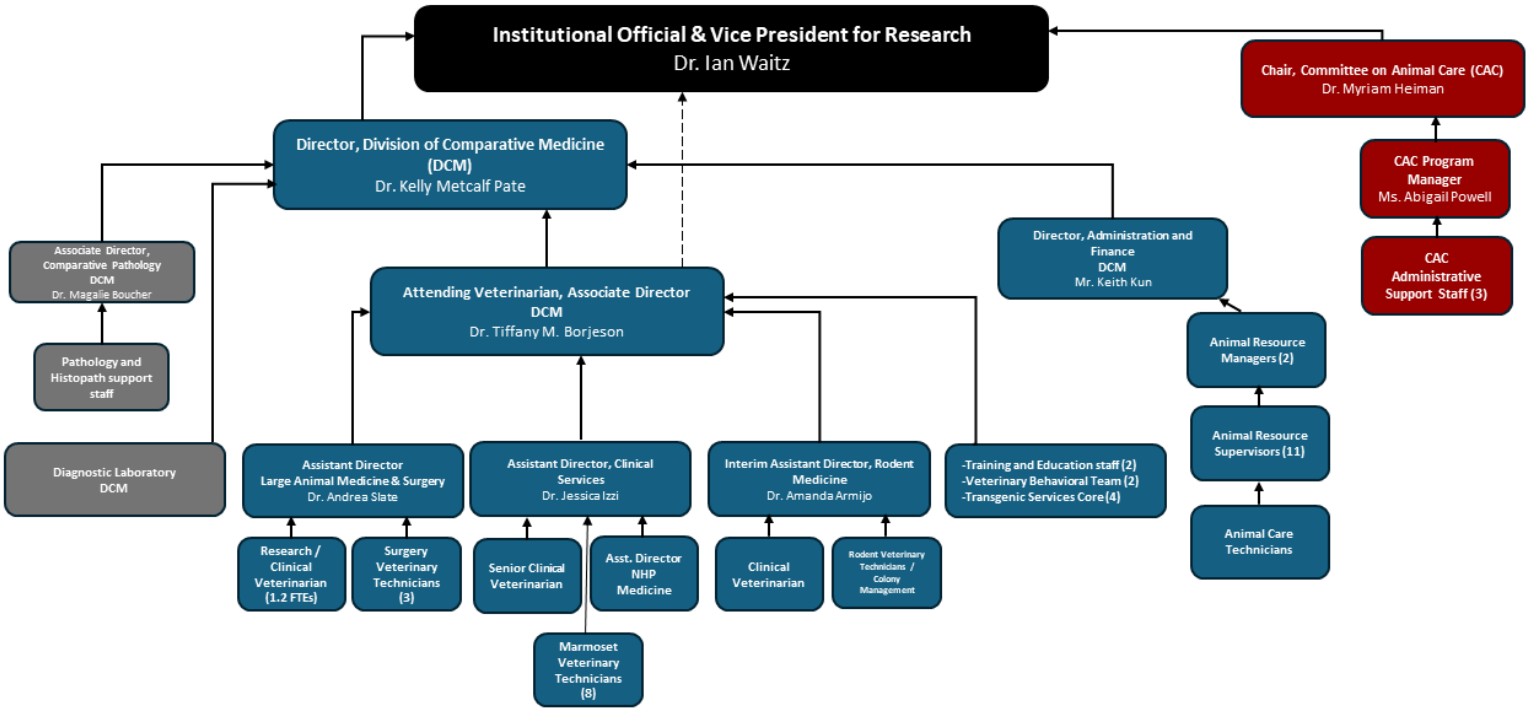
Do not hesitate to contact us whenever we can be of assistance to you. We trust this handbook will aid you in the responsible conduct of your research and teaching with animals at MIT.

Kelly A. Metcalf Pate, DVM, PhD, DACLAM

Director, Division of Comparative Medicine
Dorothy W. Poitras Associate Professor of Biological Engineering

Chapter 1: Overview

Division of Comparative Medicine (DCM) Organizational Chart



Division of Comparative Medicine (DCM) Contact Information

DCM staff assists in the procurement, care, and use of all vertebrate animals by researchers at MIT/WIBR, and provides training to all individuals working with animals. The Administrative Offices of DCM are located in 16-825 (fax 617-258-5708) and 16-849 (fax 617-252-1882).

Name:	**Position:**	**Email:**	**Contact for help with:**
Armijo, Amanda	Clinical/Research Veterinarian	aarmijo@mit.edu	
Borjeson, Tiffany	Associate Director for Clinical Services/Attending Veterinarian	borjeson@mit.edu	
Boucher, Magalie	Comparative Pathologist & Assistant Director, Comparative Pathology	boucherm@mit.edu	
Fabian, Niora	Research/Clinical Veterinarian	nfabian@mit.edu	
Fox, James	Founding Director/ Professor	jgfox@mit.edu	
Goodroe, Anna	Assistant Director for NHP Medicine	agoodroe@mit.edu	
Izzi, Jessica	Assistant Director for Clinical Medicine	jizzi@mit.edu	
Jackson, Martina	Research/Clinical Veterinarian	martinaj@mit.edu	
Kun, Keith	Director, Administration & Finance	kkun@mit.edu	
Mathieu, Erin	Manager, Animal Resource Program	mathieue@mit.edu	
Metcalf Pate, Kelly	Director, Division of Comparative Medicine	kpate@mit.edu	
Simmons, Taylor	Clinical/Research Veterinarian	taylormc@mit.edu	
Slate, Andrea	Assistant Director for LA Medicine & Surgery	aslate@mit.edu	
Zhang, Emily	Clinical/Research Veterinarian	eqzhang@mit.edu	

Moira List Title	Members	Email:	Contact for help with:
Animal Import-Export	DCM Import Export Coordinator	animal-import-export@mit.edu	Animal importing not from approved vendors/exports
Animal Requests	DCM Import Export Coordinator	animalrequests@mit.edu	Animal ordering from approved vendors (CRL, Taconic, JAX)
CJ Transfers	E25, E17/18, 46NHP Area supervisors, veterinarians, & LA vet tech supervisors	cj-transfers@mit.edu	Marmoset relocations
CJ Vet Staff	LA Vets, surgical techs, & LA supervisors	cj-vetstaff@mit.edu	Veterinary questions/concerns with the marmosets
DCMers	All DCM staff	dcmers@mit.edu	
DCM Access	Animal Resource Manager & Education and Outreach Specialist	dcmaccess@mit.edu	Facility Access requests or leaving MIT notification
DCM Area Supervisors	Facility Area Supervisors	dcmareasupervisors@mit.edu	Facility issues/questions
DCM Diagnostic Lab	Diagnostic Lab Team	dcm-diagnosticlab@mit.edu	Diagnostic Inquiries
DCM Director	DCM Director & her administrative team	dcmdir@mit.edu	Photography requests for publication & Director meeting scheduling
DCM Hazards	DCM Hazard Coordinators	dcmhazards@mit.edu	Hazard/Containment Training & Questions
DCM Large Animal Vet Techs	Surgical technicians, behavior technician, and their supervisors	dcm-largeanimalvettechs@mit.edu	Large animal clinical cases (excluding marmosets)
DCM Marmoset Techs	Marmoset technicians	dcm-marmtechs@mit.edu	Marmoset questions/concerns
DCM Pathology	Pathology group and their supervisors	Dcm-pathology@mit.edu	Pathology Inquiries
DCM Pharmacy	DCM Pharmacy Technicians and their supervisor	dcmpharm@mit.edu	Pharmacy requests
DCM Post Doc	DCM Post-doctoral candidates	dcm-postdoc@mit.edu	

Moira List Title	Members	Email:	Contact for help with:
DCM Relocation	Rodent Area Supervisors	dcmrelocation@mit.edu	Rodent relocations
DCM Rodent Vet Tech	Rodent vet techs and their supervisors	Dcm-rodentvettechs@mit.edu	Technical services requests, rodent clinical cases, colony management
DCM Scheduling	Surgical Techs, Assistant Manager for Large Animal Technical Services, & LA Vets	dcm-scheduling@mit.edu	Scheduling Large Animal Surgeries & OR Spaces
DCM Training	Education and Outreach Team	dcmtraining@mit.edu	Training requests or questions
DCM Vet Tech	Small/large animal veterinary technician supervisors	dcmvettech@mit.edu	Questions relating to vet techs or veterinary/experimental matters; anesthesia machine oversight
Marmoset Training	Assistant Manager for Large Animal Technical Services and their designee	marmosettraining@mit.edu	Requests for Marmoset Training

Committee on Animal Care (CAC)

The Committee on Animal Care (CAC) is responsible for overseeing all vertebrate animal work at MIT/WIBR. **CAC approval is required in advance for all vertebrate animal use and for authorization of specific individuals to work with animals.** The Animal Welfare Act and Public Health Service Policy mandate the existence, structure and function of the Institutional Animal Care and Use Committee (IACUC). At MIT, the IACUC is called the Committee on Animal Care (CAC), and its members are appointed by the Institute's President. The CAC has at least five members and attempts a balance between scientists and nonscientists. There is at least one member who is not affiliated with MIT.

The CAC was established to ensure that all MIT and WIBR researchers working with animals or making funds available to support other researchers' work with animals comply with **federal, state, local, and institutional regulations** on animal care. To that end, the CAC inspects animals, animal facilities and laboratories, and reviews all research and teaching exercises which involve vertebrate animals or other tissues before activities are performed.

All research and teaching exercises involving animals must obtain prior approval from the CAC.

See the CAC website or contact the CAC office (cacpo@mit.edu) for detailed information on Principal Investigator responsibilities, guidance for protocol preparation, information on

approval of collaborations within and outside of MIT/WIBR ("Collaborations" policy), changes to an approved protocol, a detailed explanation of the CAC review process, and using laboratory space outside of DCM facilities for animal experiments ("Laboratory Space as Animal Use Area" policy).

The CAC website may be utilized to obtain information on meeting dates and submission schedules, CAC policies, and protocol application forms for describing animal use in research studies. **MIT Touchstone Authentication (MIT Kerberos ID and/or MIT Certificates) is required to access the website.**

CAC Contact Information

The CAC Protocol Office is located in 16-408 (email: cacpo@mit.edu).

Name	**Position**	**Email**	**Contact for help with:**
Borjeson, Tiffany	Associate Director for Clinical Services/Attending Veterinarian	borjeson@mit.edu or mit-av@mit.edu	General animal use program concerns
Gillis-Smith, Sarah	CAC Director of Outreach and Compliance	sarahgs@mit.edu	Protocol/addendum preparation and policy guidance
Heiman, Myriam	CAC Chair	cac-chair@mit.edu	General animal use program concerns
Metcalf Pate, Kelly	DCM Director	kpate@mit.edu or dcmdir@mit.edu	General animal use program concerns
Powell, Abigail	CAC Program Manager	ascherer@mit.edu or cacpo@mit.edu	Protocol/addendum preparation, status updates, and policy guidance
Waitz, Ian	CAC Institutional Official, Vice President for Research	mit-io@mit.edu	General animal use program concerns
Moira List Title	**Members**	**Email:**	**Contact for help with:**
CAC Protocol Office	Abigail Powell Nicole Cardona Kris Cundari Desiree Meuse Spencer Steigman	cacpo@mit.edu	All CAC questions, protocol submissions, preparation, or policy guidance

Animal Welfare Contacts

For any critical/immediate animal emergency, please call the applicable facility's clinical veterinarian. Names and contact information are posted within the facility, including on-call veterinary contact phone number(s). Veterinary staff are available 24 hours a day/7 days per week for animal emergencies. Alternatively, DCM's main number (617-253-1757) may be utilized to contact the answering service, if needed.

Reporting Animal Welfare Concerns ("Whistleblower" Policy)

The "Whistleblower" Policy is posted at DCM facility entryways, periodically on DCMtv, at the CAC website, and to MIT's online news (by request, in March and September).

The Committee on Animal Care (CAC) and the Vice President for Research welcome any information which would aid our efforts to assure the humane care of research animals used at MIT and the Whitehead Institute for Biomedical Research. Anyone with concerns about the welfare of animals involved in research at MIT should report those concerns to the CAC even if the individual is not directly involved in the research.

Established to ensure that MIT researchers working with animals comply with federal, state, local and institutional regulations on animal care, the CAC inspects animals, animal facilities, and laboratories, and reviews all research and teaching exercises that involve animals before experiments are performed.

If you have concerns about animal welfare, please contact the Committee on Animal Care (CAC) by calling 617-324-6892, or send your concern in writing to the CAC Office (room 16-408, note CAC Office members work primarily remotely so responses to concerns may be delayed if left via this method), by email at cacpo@mit.edu. The issue will be forwarded to the Chair of the CAC and the Attending Veterinarian.

You may also contact any of the following:

- Vice President for Research: 617-523-9584, mit-io@mit.edu
- Director of the Division of Comparative Medicine: 617-685-1830,dcmdir@mit.edu
- Associate Director for Clinical Services: 617-444-9434, mit-av@mit.edu
- CAC Chair: 617-324-6892, cac-chair@mit.edu
- Anonymous reporting hotline: https://hotline.mit.edu/

All concerns about animal welfare will remain confidential; the identity of individuals who contact the CAC with concerns will be treated as confidential and individuals will be protected against reprisal and discrimination consistent with MIT Policies. The Committee on Animal Care will report its findings and actions to correct the issue to the Vice President for Research, the Director of Comparative Medicine, the individual who reported the concern (if not reported anonymously), and oversight agencies as applicable.

MIT's anonymous reporting hotline that is hosted and maintained by a third-party vendor, Ethicspoint. To get more information or file a report, go to https://hotline.mit.edu/

Animal Facilities

DCM oversees the animal facilities at MIT and WIBR. For security reasons, all facilities are restricted to authorized personnel only, and photography and videos are only permitted under limited circumstances.

Introduction to DCM Facilities

DCM houses a number of different animal species in its animal facilities.

Personnel may only enter a DCM animal facility with explicit CAC and DCM approval. DCM will not allow anyone who has entered another institute's animal facility to enter a DCM animal facility for 48 hours after they have been in the outside facility.

Policy on Entry for Unauthorized Personnel/Children/Visitors

Unauthorized personnel, including children, are strictly forbidden from entering any animal facility. Permission and arrangement for tours through MIT/WIBR animal facilities are arranged with the DCM Director's office. Authorization for guest entry for reasons unrelated to animal experiments requires written approval by the Principal Investigator (PI) and DCM's Animal Resource Managers. Visiting researchers involved in animal experiments should submit a Visiting Personnel Addendum (VPA) to the CAC office.

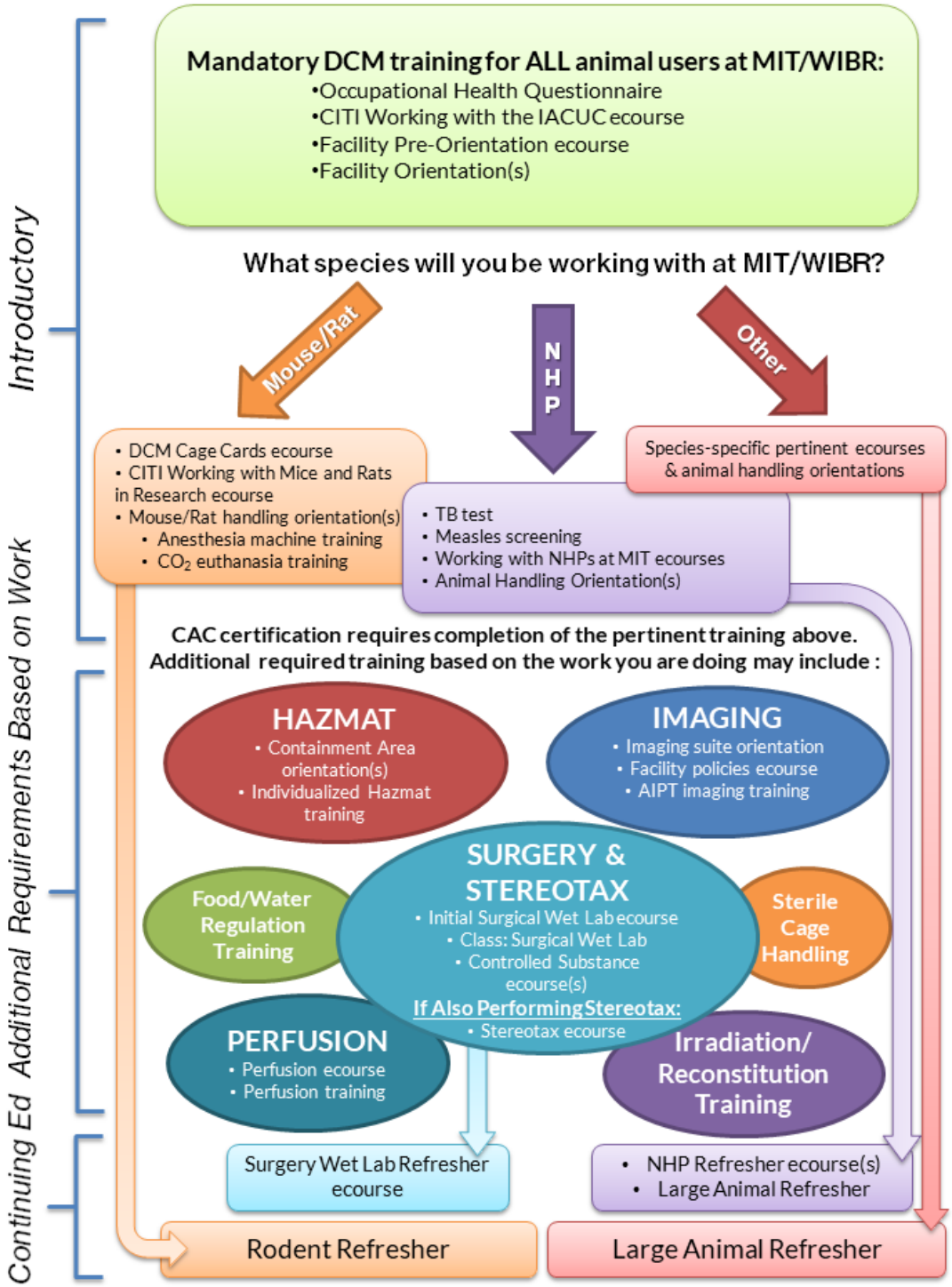
Unauthorized entry into the animal facilities may result in the interference of research activity, inadvertent exposure of trespassers to hazardous substances, transmission of infectious diseases to animals within the facilities, vandalism, theft or loss of animals.

Policy on Photos/Videos

Photographs or videos of animals, animal facilities, or experiments are not permitted. Approval for this requires explicit, written permission from DCM and the CAC. Please see CAC policy "Photographs or Videos of Animals or Experiments" on the CAC website.

Chapter 2: Training

Training Tree



Registration for all training is completed through the MIT Learning Center.

Visit: https://comp-med.mit.edu/resources/required-training for instructions on how to affiliate with the appropriate Training Groups and register for training. Pertinent contact information will be listed in individual course descriptions.

For any questions on training, or problems with the Learning Center, contact: dcmtraining@mit.edu

CAC Certification

The United States Department of Agriculture (USDA) enforces the Animal Welfare Act (AWA), and the Office of Laboratory Animal Welfare (OLAW) enforces Public Health Service Policy (PHS Policy). USDA and OLAW require MIT's Committee on Animal Care (CAC) to verify that all investigators, staff, and students using animals in research or teaching have received appropriate training. Of particular concern is that all animal users understand animal welfare and practice humane treatment of animals. DCM and the CAC have developed a certification training program to assist investigators in understanding what is expected when working with animals at MIT/WIBR.

PIs must complete the CAC certification training program for use of animals in teaching or research, regardless of whether they will handle animals. All new PIs will thus need to complete CAC certification. All PIs with approved protocols using mice or rats are also required to periodically attend Rodent Refresher training; this includes PIs who are not doing hands-on work with mice or rats. More details, including details on possible exemptions, are available in the CAC Policy "Principal Investigators."

New Personnel Addendum

To be added to an animal care and use protocol, required training must be completed and CAC certification must be obtained. Complete the "New Personnel Addendum" (NPA) form and submit it to the CAC office only **after** the required training courses in the MIT Learning Center have successfully been completed additional paperwork is required of undergraduates (see the CAC policy on "Undergraduate Participation in Research"). The applicant will be notified of approval status within a week of submission in most cases. However, in some cases, clarifications may be sought by the CAC before approval is granted.

When approval has been received, email dcmaccess@mit.edu to request facility access. The email must include your name, Kerberos, MIT ID#, and facility/facilities to which access is being requested. **You must request your own facility access; if you do not, you are liable to lose CAC certification and be required to re-take all training courses.** It is expected that, even after NPA approval, additional training and/or oversight may be necessary.

Written approval from the CAC and individual card access are required before the applicant may enter any animal facility or begin using animals.

CAC approval is protocol-specific, so a new NPA is required to join additional protocols.

Training documented on a previously submitted NPA will not need to be repeated, unless it has been more than six months since the investigator last worked with animals on campus, will be working with a different species than previously approved for, or will be working in a different animal facility.

Affiliating with Training Groups

Affiliate with the appropriate Training Groups in the MIT Learning Center: https://atlas.mit.edu/atlas/Home.action in order to register for DCM-specific training.

Step-by-step instructions can be found on the DCM and CAC websites: **At minimum, you will need to join one group based on the facility in which you will be working and species with which you will be working**. A separate orientation is necessary for each facility you plan to use.

If you will be working exclusively with post-mortem tissues, join the "Ex-Vivo Studies" Training Group under "Additional Required Training".

You will also need to join additional Training Groups for training in surgery (including perfusion), containment/hazmat, food/water regulation, imaging, and irradiation, as applicable.

Online Training

Online training is broken down into two components: CITI training and DCM e-courses. CITI courses cover fundamentals of working with the IACUC and species-specific information. DCM e-courses cover details specific to working in MIT/WIBR animal facilities. The pertinent courses are assigned based on the Training Groups joined.

Occupational Health

All persons that come in contact with vertebrate animals used in research or teaching, or their tissues, must submit an "Occupational Health Questionnaire" (OHQ) to MIT Medical. DCM also encourages lab members who do not have direct contact with animals but are subject to indirect exposure via procedures performed in the lab to also complete an OHQ. An occupational health provider at MIT Medical will review your OHQ and provide follow-up consultation, as warranted.

Persons that will come into contact with nonhuman primates or biological samples from nonhuman primates (tissues and/or bodily fluids) are required to undergo tuberculosis testing. This documentation must be current within the previous calendar year, and personnel with continued exposure to NHPs or their biological samples are required to undergo yearly testing. The tuberculosis assessment at MIT Medical includes a discussion of herpes B virus risk. Persons working with NHPs are also required to demonstrate immunity to measles virus through a one-time measles screening. Appointments may be made with MIT Medical's Occupational Medicine and Employee Health Service to obtain TB and measles immunity screening.

Animal Facility Orientations

Orientation is necessary for each facility you plan to use. Orientations review standard operating procedures within the facility, including: proper PPE usage, facility traffic patterns, basic husbandry practices, and cage card systems for health checks, etc.

If you need access to an additional facility after your NPA is approved, but are not joining a new protocol or working with a new species, affiliate with the appropriate new Training Group and register for an orientation in the new facility. Previously completed training requirements (e.g. animal handling training) will be recognized as having been fulfilled. The CAC does not need to be notified of these situations (no signature or updated paperwork is required).

The DCM Facilities Pre-orientation e-course must be completed prior to your scheduled facility orientation.

Species-Specific Training

Personnel are required to receive training from DCM staff in basic handling of each species they are working with.

Protocol-Specific Training

Investigators are required to read the CAC-approved protocol(s) they are joining and receive training in protocol-specific techniques from an experienced fellow lab member or a DCM staff member.

Visiting Personnel Participation in Research

Visiting personnel intending to only work with animals under direct supervision of a senior, CAC-certified laboratory member, and whose tenure in the lab will be brief, may submit a "Visiting Personnel Addendum" (VPA) form in lieu of completing CAC certification. See the CAC website ("Training Requirements for Visiting Personnel") for more information.

Tissue Use/Tissue Harvest Program

Persons who will work solely with postmortem tissues provided by others need to join the Ex-Vivo Studies Training Group in the MIT Learning Center, and submit an NPA request to the CAC. The NPA should clarify that the person will only work with tissues. Each PI must have their own Tissue Harvest Exemption Form for the application to use animal tissues or bodily fluids. For those working with NHP tissues/biological products, additional requirements, as indicated by OHS may need to be completed as outlined above.

Additional Required Training from DCM

Anesthesia Machines

Rodent anesthesia machine use is discussed in person during basic handling sessions which are required of all investigators using mice and rats. Personnel who would like additional training may register for an optional Anesthesia Wet Lab (EHS01056c). Training for inhalant anesthesia units utilized in large animal species are provided as part of the surgical training required for all non-rodent mammalian species.

DCM offers the use of inhalant isoflurane machines within all animal facilities. IMPAC6 and COMPAC5 machines are provided for rodents, and standard calibrated anesthetic vaporizers are utilized for large animal species. In addition, many investigators have their own anesthesia machine units in their lab space. All anesthesia units must be serviced annually, and adequate scavenging of waste anesthetic gas and disposal of expired or unused anesthetic agents, such as isoflurane, should be in place. It is important to use these machines safely to eliminate exposure to waste gas. DCM must be notified of all new anesthesia machine installations. Contact dcmvettech@mit.edu.

Euthanasia

Investigators may euthanize animals only if they have been trained during a species-specific training course or by one-on-one instruction from a veterinary staff member. Euthanasia methods must match those described in the relevant CAC-approved protocol. Animals are not to be euthanized in the animal room itself unless the room has been placed under quarantine. Instructions for CO_2 euthanasia are posted at the euthanasia stations in procedure and necropsy rooms; the instructions also must be posted in your lab if you have CAC permission to euthanize animals by that method outside of a DCM animal facility. Contact DCM Training for a copy of the most up to date CO_2 euthanasia SOP.

Hazardous Material ("Hazmat") Use in Animals

Approval Process

Approval to use hazardous materials in animals must be approved by the CAC. After the CAC review, MIT's Environment, Health and Safety Office and/or MIT/WIBR's Environmental Health and Safety Office (referred to henceforth as MIT/WIBR's EHS) will also review the materials and provide a hazard-specific Risk Assessment (RAF) to the DCM Hazards Coordinator. After the CAC component is approved and near the time the lab personnel wants to begin their experiments, study personnel **must** contact the DCM Hazards Coordinator (dcmhazards@mit.edu) to request hazard-specific training for the hazard they wish to work with. The DCM Hazards Coordinator or their designee will request the EHS review of the material at this time and the RAF will be used to train the study personnel. **Only after the RAF for a given hazmat have been completely approved and processed by EHS and DCM, and training has been completed for personnel, may a hazard be used in animals, with prior notification.**

Training of Hazmat Use in Animals

When experiments using a hazmat are to begin, researchers **must** request and receive training in the animal facility about specific safety guidelines from the DCM Hazards Coordinator or their designee. This training should occur shortly before the study is initiated; retraining may be required if the study is delayed. Note that no personnel can enter the containment areas without specific training from the DCM Hazards Coordinator or their designee, and there is no shadowing of unapproved personnel.

Animal Imaging

There are two facilities on campus for animal imaging studies.

For rodents, the Koch Preclinical Modeling, Imaging, and Testing (PMIT) Core Facility ("Imaging Area" or IA) is located within the 76 facility and contains integrated housing for rodents used in imaging experiments. Koch Institute faculty member Dr. Aurora Burds Connor supervises the IA with onsite management by the Preclinical Modeling, Imaging, and Testing team (AIPTcore@mit.edu). Virginia Spanoudaki (vspan@mit.edu) heads the Preclinical Imaging & Testing Facility and is the point of contact for use of the equipment. More information on the services provided can be found at https://ki.mit.edu/sbc/aipt . They can provide fully assisted services, trainings for independent instrument use and study design consultation on whole animal imaging using bioluminescence, fluorescence, ultrasound & photoacoustic, MRI and microCT technologies; everyone using the IA independently must be trained. Assisted imaging experiments are conducted under the facility's CAC approved protocols. The CAC needs to pre-approve imaging studies, and housing space is assigned by DCM.

All personnel performing imaging in building 76 should affiliate with the "76 Imaging Suite" Training Group in the Learning Center and complete the associated training requirements.

For large animals, the Martinos Imaging Center in 46, directed by Dr. John Gabrieli, has a 9.4 Tesla MRI for animal studies. This area is managed by Steve Shannon (sshannon@mit.edu); more information on the MRI can be found at the website http://mcgovern.mit.edu/technology/martinos-imaging-

Investigators desiring to perform experiments using this MRI should first discuss plans with their facility veterinarian, who can offer guidance for obtaining CAC approval as well as the practical aspects of transporting and monitoring animals to be imaged.

Irradiation/Reconstitution in Mice

Myoablation, or irradiation of rodents, is performed such that they will accept transplanted cells. Details regarding dose, frequency, and time-to-reconstitution must be indicated in the protocol and be approved by the CAC. This procedure must also indicate off-target effects, such as septicemia, failure of reconstitution or graft-versus-host disease and/or other immune-mediated issues related to the implanted cell type. Personnel wanting to conduct irradiation and/or

reconstitution studies in rodents must review the CAC Policy on this topic, Irradiation (Myoablation) and Reconstitution Studies in Rodents.

Once approved, users wanting to irradiate animals must affiliate with the "Req For: Irradiation/Reconstitution" Training Group in the Learning Center and complete the associated training requirements prior to obtaining access. Investigators must meet with the DCM veterinary staff to discuss requirements for transport, irradiation and post-procedural monitoring of mice. Additional training to obtain independent access to either the cesium-source irradiator or x-ray irradiator must be completed with a member of EHS.

Surgery

The CAC and DCM require that all individuals who will be or are performing surgery (survival or non-survival), as well as those performing post-operative surgical evaluation of animals, are trained. This training is accomplished in several ways, including the e-course and surgical wet lab offered by DCM. The online training is then re-completed once every three years. Additional training by a senior lab member proficient and independent in the procedure or a DCM member (veterinarian, training coordinator, in vivo technical support personnel) may be required. Surgical training is not required for those investigators performing procedures on dead animals. Failure to complete initial surgery training and refresher training every three years will be discussed by the CAC and may result in suspension of the personnel or the surgical component of the protocol.

New surgeons must complete DCM surgical training (e-course and Surgery/Post Op Care Wet Lab) and receive training from a senior lab member or DCM staff proficient in the technique(s) before performing surgeries.

- Affiliate with the "Req for: Surgery/Post-Op Care" Training Group in the MIT Learning Center. Initial training consists of a pre-requisite e-course and attendance at Surgery/Post Op Care Wet Lab. Individuals are then required to complete online refresher training every three years to remain compliant.
- The Surgery/Post-Op Care Wet Lab provides a hands-on overview of surgical preparation and techniques including aseptic prep of instruments and the animal, anesthesia, incision and closure techniques, and peri- and postoperative analgesia, maintenance, and monitoring methods.
- For non-rodent species, DCM veterinary staff can provide individual training and oversight, and surgeries are performed only in DCM-managed surgical suites.

Undergraduates must have direct supervision from their immediate supervisor during surgery.

A surgical wet lab designed for surgeons working with zebra finches is offered by DCM.

Special Diets, Special Water, Food and Water Regulation

All protocols involving special diet feeding, special water feeding, and food or water

regulation must be scientifically justified and approved by the CAC. Personnel conducting food or water regulation in rodents must affiliate with the "Req for: Rodent Food & Water Regulation" Training Group in the MIT Learning Center and complete the associated training requirements. Note that if animals are provided investigator-fed special diet or special water are found with low food or water they will be provided standard rodent chow and/or a water bottle at the discretion of the ACT, without prior notification of the researcher. Animals found on regulation of food or water and not appropriately provided food or water per protocol may be provided standard rodent chow and/or a water bottle at the discretion of the vet staff after notification to the researcher.

For regulating species other than rodents, the CAC has policy statements, and standard operating procedures are available. Contact the CAC (cacpo@mit.edu) or a DCM veterinarian for more information.

Continuing Education

Annual Refreshers (Mandatory)

The Rodent Refresher is an annual continuing education seminar which highlights and reinforces basic content when working with rodents, highlights common mistakes or drift from the standards that have the potential to occur, and provide updates to any changes in standard practices within the facility.

All investigators working with mice, rats, hamsters, and gerbils including PIs are required to attend a Rodent Refresher session once per calendar year and attendance at these seminars is required to maintain facility access. Introductory hands-on training does not fulfill this obligation, and attendance is required even in the year that you begin work at MIT. PIs who do not work directly with animals (listed only as supervisory under protocol responsibilities) are required to attend a Rodent Refresher session once every three years.

The Large Animal Refresher is an annual continuing education seminar which highlights and reinforces basic content when working with USDA covered species, including NHPs, swine, rabbits; as well as finches where relevant changes or updates should be conveyed to the research community at-large. Large Animal Refresher is required once per calendar year for all individuals working with USDA covered non-rodent species and/or finches including PIs who do not work directly with animals (listed only as supervisory under protocol responsibilities.)

Dates, times, and locations are posted in the MIT Learning Center, on DCMtv, and in monthly lab rep meeting notes. **Investigators who fail to attend a yearly session will lose access to the animal facility and CAC certification is revoked. To regain access, investigators are required to re-complete *all* of their introductory training and orientations. Work with animals may not resume until CAC recertification is complete.**

Additional Continuing Education for Researchers Working with Non-Human Primates

Research staff and personnel working with NHPs must complete the "Working with NHPs at MIT – Refresher" e-course on an annual basis. This course covers zoonoses, behavioral management, food and water regulation, and emergency procedures. Triennially, researchers must complete "Working with NHPs at MIT: Course 3" which covers pertinent information concerning surgery in nonhuman primates.

Researchers working with species other than those named above are required to complete species-specific CITI training courses every three years.

Monthly Lab Rep Meetings

Monthly Lab Rep meetings create a forum where DCM personnel and animal users can discuss facility and animal-related issues. Each laboratory group should designate a person to be the lab liaison (laboratory representative, or lab rep) to DCM and attend the monthly meetings.

During the meeting DCM will present pertinent information to the group regarding animal-related topics and/or facility-related topics at MIT (e.g., room renovations, changes in husbandry-related items, updates to policies or practices). At this meeting, the lab reps have the opportunity to present issues or concerns from their lab groups. Meeting minutes will be emailed to the designated lab rep. It is the lab rep's responsibility to disseminate this information to all the animal users within their lab group.

Meetings are held monthly on a set schedule for each animal facility. Facility and researcher email lists will be used to notify relevant attendees; please email dcm-info@mit.edu if you would like to be added to the list.

Lab Rep meeting schedule:
68N and 68S – Second Tuesday of every month at 11:00 am in 68-180
46Tg and 46SA – Second Thursday of every month at 11:00 am in 46-7001
76 – Third Tuesday of every month at 11:00 am in 76-659
WIBR – Last Tuesday of every month at 11:00 am in WI-227
46NHP – One Monday per month at 11:00 am in 46-7011
56, E17/18, E25 – Scheduled as needed

Chapter 3: Occupational Health and Safety

Occupational Health Program (OHP) for Personnel with Animal Contact

The occupational health program for personnel with animal contact is coordinated through a partnership between the Division of Comparative Medicine, the Committee on Animal Care, the Environmental Health and Safety (EHS) Office, the MIT's Occupational Health service, those personnel who work with animals and MIT's upper administration.

Researchers, employees, students, and visiting scientists that have *any* animal contact are required to submit an "Occupational Health Questionnaire" (OHQ) to MIT Medical prior to working with animals or gaining facility access. The OHQ can be submitted electronically (preferred) or printed and sent, or hand delivered to the Occupational Health department at MIT Health. Occupational Health professionals perform a risk-based assessment for occupational exposures based on the species you will be using. An in-person exam/evaluation may occur to obtain baseline data. Additional testing, diagnostics, and/or vaccinations may be required to obtain Occupational Health clearance. Once the assessment has been completed, enter the date of the OHQ on the "New Personnel Addendum" or "Visiting Personnel Addendum". **Note that the OHQ contains private medical information and should NOT be sent to DCM or the CAC office.**

Respirator fit testing by MIT Medical and MIT/WIBR's EHS is required for those individuals deemed by Occupational Health evaluation to require respiratory protection when working with animals, seeking allergy control or when indicated on a "DCM Protection/Control Required for Hazard Use in Animals" form based on experimental hazards.

For any personnel that have contact with live nonhuman primates or may come in contact with unfixed 'fresh' samples or frozen biological samples from nonhuman primates (e.g., tissues, biopsies, blood, urine, etc.) must undergo education about zoonotic risks of working with these animals and/or samples. Samples preserved in formalin are not a known risk. Personnel working with nonhuman primates and/or their tissues as outlined above are given information about tuberculosis and herpes B virus as part of CAC web-based training, facility and veterinary orientation sessions, and tuberculosis screening conducted by MIT Medical.

Research staff working with hazardous materials (hazmats - including biohazardous agents, chemical hazards, or radioactive compounds) must participate in the appropriate Occupational Health program as designated by the relevant MIT/WIBR's EHS. Please see above [Hazardous Material (hazmat) Use in Animals] for complete details on submission and/or approval pathways for the use of hazmats in animals. Note that review by MIT/WIBR's EHS and CAC must occur, and written approval must be granted **before** starting work with hazardous materials in animals. Additionally, the safety precautions and training of the agent **must be discussed with DCM's Hazards Coordinator, prior to hazardous material work with the specific agent in animals can start.**

Personnel Protective Equipment

DCM requires specific PPE to be worn upon entry into the facility and when working with animals and/or hazards in animals. These requirements are posted at the entry doors to the facility and/or posted at the level of the specific room or cubicle, if additional PPE requirements are needed. Cloth lab coats should be used when available. No laboratory-owned lab coats are allowed within the animal vivaria.

If working with animals within the laboratory space, a laboratory-owned lab coat (laundered after use with animals and/or disposable), gloves and a mask are required when working with animals in laboratories outside of DCM facilities.

Physical Injury

Avoidance of exposure to radiation ("x-rays"): Diagnostic radiographs will be performed by the veterinary staff. If radiographs are part of an experiment, the procedures and training should be described in the CAC animal use protocol. Persons that use radiation experimentally or diagnostically must be adequately trained by staff of MIT/WIBR's EHS Radiation Protection Program and be monitored by dosimetry. Protection from radiation is primarily a function of radiographic technique, and guidelines for minimizing exposure to radiation, including the use of lead gowns and gloves, are available from DCM veterinary staff.

Safe use of volatile (gas) anesthetics: Inadvertent exposure of personnel to waste anesthetic gases has been associated with liver abnormalities and problems with fertility; the potential for unknown health effects is a further concern. Volatile anesthetics can only be used in conjunction with adequate scavenging of waste gases, to include precision vaporizers on anesthetic machines and anesthesia circuits fitted with scavenging systems or the use of volatile anesthetics in a ducted biosafety cabinet, chemical fume hood, or slot hood. Ether is prohibited because of fire and explosion dangers and the availability of safer alternatives. MIT/WIBR's EHS offers isoflurane exposure monitoring to provide assurance that work practices (using isoflurane with a bell jar, slot or chemical fume hood, vaporizer) are not exposing lab personnel to isoflurane.

Accidents: Any accidents resulting in physical injury to personnel must be reported immediately to their supervisor and MIT Medical. An EHS Report of Injury must be completed and sent to MIT/WIBR'S EHS on the MIT's Atlas website by the injured person's supervisor. Specific treatment protocols for bites, scratches, or splashes from nonhuman primates or scratches from potentially contaminated equipment are in effect which require the specific identification of the animal involved. This is also required of those working with fresh biological samples or freshly frozen biological samples of nonhuman primates. If necessary, DCM staff will assist the injured person in receiving medical attention at MIT Medical. The initial report filed with the MIT/WIBR'S EHS offices documents the accident and ensures that further treatment for problems related to the accident will be provided.

Bites and scratches: Animal bites can cause severe mechanical damage and, in some instances, pose a serious threat of zoonotic disease transmission. Implications for disease transmission are

greatest for bites from rats, ferrets and nonhuman primates. Every incident must be reported to the individual's supervisor and an accident report filed with MIT/WIBR'S EHS. **All persons that receive an animal bite, except if bitten by a mouse, should be evaluated by MIT Medical. If bitten by a mouse, you should go to MIT Medical if the mouse was injected with a hazardous material and/or if the bite involves a joint.** Rabies is not a risk with the species used in DCM animal facilities.

Nonhuman primates: Guidelines are in place for persons bitten, scratched, wounded, or splashed (on a mucous membrane) by a nonhuman primate or equipment contaminated by a nonhuman primate. Once the wound has been disinfected, the affected person must go to MIT Medical for evaluation of the risk of herpes B virus infection (if a macaque is involved). This is also required of those working with fresh biological samples or freshly frozen biological samples of nonhuman primates. Nonhuman primate "Bite Kits" with directions for wound/splash treatment and supplies are located inside each nonhuman primate holding room or vestibule, throughout the animal facility where nonhuman primates are housed, and in each laboratory where nonhuman primates are taken for training. Annual testing for herpes B virus occurs in all macaques. If needed, additional testing or repeat viral testing may occur if a macaque is involved with an human exposure. Persons working with New World nonhuman primates, such as common marmosets, should follow the bite kit instructions for scrubbing and also report to MIT Medical; however, herpes B virus transmission is not a concern.

Needle sticks: **Needles used for injection should never be recapped after use because this increases the risk of an inadvertent skin puncture.** All "sharps" (needles, scalpel blades, sharp glass, etc.) must be placed in a sharps container (marked with the biohazard symbol) for proper disposal. Wounds from needle sticks should be scrubbed with water and disinfectant (chlorohexidine or povidone iodine) and then be reported to your supervisor. Persons injured while working with nonhuman primates or human tissues/biological fluids must report to the MIT Medical for evaluation as described above.

Allergies

Contact with various animal species, especially rodents and rabbits, is known to result in allergies in susceptible humans; allergies affect approximately 10-20% of people who work with animals. Handling animals in a ducted biosafety cabinet (BSC), an Animal Transfer Station (ATS), fume hood, or a laminar flow workstation may significantly reduce exposure to animal allergens and is required for all personnel working with animals. Should this not be possible, personnel must wear a face mask when handling animals outside the BSC, ATS or fume hood. MIT's MIT/WIBR'S EHS conducts a respirator fit program for individuals which may experience a high risk of aerosol exposure to offending allergens or other hazards.

Zoonoses of Laboratory Animals

Zoonoses, as defined by the World Health Organization in 1958, are "…diseases and infections which are naturally transmitted between vertebrates and humans." These infections may be

caused by viral, bacterial, fungal, protozoal and helminthic agents. Many of the characteristics of the laboratory animal setting, including strict control over vendor choice, quarantine testing, embryo transfer rederivation of rodents, housing and husbandry practices, robust disease surveillance, use of personal protective equipment, restraint devices, safe handling practices, and the institutional occupational health and safety program are designed to minimize the occurrence of zoonotic infections. Engineering and programmatic measures notwithstanding, the most important protection against zoonoses is strict adherence to established safety protocols by all persons having contact with animals and their tissues. These measures and standards are not only meant to protect humans, as some diseases can be transmitted from humans to animals (e.g., tuberculosis from humans to macaques). Other sources of zoonotic disease are those spread indirectly from animal to human by arthropods, inanimate objects, e.g., caging, cell cultures, biologics and organ transplants, i.e., xenozoonoses. Thus, insect control, standardized sanitation practices, and testing of biologics also help preclude zoonotic transmission of diseases

The occupational health staff of MIT Medical can answer questions pertaining to human infection with zoonotic organisms. It should be recognized that any animal may carry organisms which have the potential to potentially cause a disease state in humans. Personnel that are pregnant or immunocompromised by disease or disease therapy should be cautious and discuss their responsibilities with Occupational Health at MIT Medical and / or their supervisors. Any individuals seeking medical care that have contact with animals or animal tissues should inform attending physicians of their occupational exposure.

Chapter 4: Animal Acquisition, Quarantine/Acclimation, and Transfers

Centralized Procurement from DCM

All animals intended for research at MIT/WIBR must be purchased through DCM and be housed in DCM-approved facilities. Offsite projects, such as custom antibody production by a commercial company, must first be approved by DCM and the CAC, and then be submitted to MIT's Sourcing and Procurement office (X37241, NE49-4122).

Ordering Animals

Investigators may receive laboratory animals from a variety of sources. **Regardless, all animal orders must be placed through DCM.**

When animals are ordered from DCM-approved vendors (i.e., Taconic Biosciences), the purchased animals will be delivered directly to a DCM animal facility, subject to space availability and animal health status. The veterinary staff routinely reviews the health reports from approved vendors.

When animals are requested from a non-approved source (i.e., academic institution) a DCM veterinarian must approve the shipping institution's health report before animals from non-approved institutions can be imported. See details below under "Gift Animal Request/Atypical Vendor".

Animals from DCM-Approved Vendors

All requests for animal purchases must be submitted by EOD (11:59pm) Tuesday the week prior to the desired delivery date. Investigators are encouraged to place orders even earlier to ensure availability of animals needed for their research, as some species and strains are sold in limited quantities. DCM will inform investigators when animals are not available or when a long backorder exists.

To place an animal order, a completed "Request for Laboratory Animal Purchase and Care" form must be submitted to DCM's Animal Purchasing Coordinator. Only one form per request should be submitted, and it must have the appropriate signatures and approved CAC protocol number before the animal order can be processed. Species/strain/stock information of animals being ordered must be approved on the CAC protocol. In addition, an account number for billing must be provided; this authorizes the expenditure of funds for the animal purchase and related care costs. The account should be among those identified in Section 2 of the relevant CAC protocol.

DCM-approved animal vendors are accredited by the AAALAC international whom assure they comply with legal requirements for animal procurement, production, and distribution. DCM selects vendors that maintain strict animal health programs, including screening of animals for

infectious agents.

List of Approved Vendors:

- Birds- Consult DCM veterinary staff
- Frog- NASCO, Rana Ranch Bullfrogs, Connecticut Valley Bio
- Hamster- Charles River Laboratories, Inc.; Inotiv
- Mouse- Taconic Farms, Inc.; The Jackson Laboratory; Charles River Laboratories, Inc.
- Nonhuman primate- Consult DCM veterinary staff
- Rabbit- Charles River Laboratories, Inc.
- Rat- Taconic Farms, Inc.; Charles River Laboratories, Inc.
- Swine- CBSET, ABI; Sinclair (mini-pigs)

Costs Associated with Ordering Laboratory Animals from DCM-Approved Vendor **can be found on the DCM website**.

Animals from Atypical Vendors (Non-Commercial, Academic, etc.)

Investigators requesting animals from non-approved vendors must complete a "Gift Animal Request/Atypical Vendor" form. To obtain this form, contact DCM's Import/Export Coordinator. The form may be submitted prior to receiving CAC approval to add a particular "gift" mouse strain to the protocol, but the mice cannot be used until CAC approval is granted; embryo transfer rederivation may also be required before the mice are released for use by the lab.

Procedure for Receiving "Gift" Animals from Atypical Vendors/Outside Institutions:

Step 1 – Submit a "Gift Animal Request/Atypical Vendor" form to the Import/Export Coordinator. The Principal Investigator (PI) or the lab's administrative officer must sign the form, and an account number must be provided for billing. Also submit the "Embryo Transfer Request and Billing" form at this time, if needed.

Step 2 – The Import/Export Coordinator contacts the shipping institution/animal source for the most recent health report. A DCM veterinarian must review and approve the health report. Note that if health reports cannot be furnished by the shipping institution or are deemed to be of an unacceptable health status for importation, the veterinarian has the right to refuse animal importation or may ask for additional testing from the source of animals prior to importation.

Step 3 – Once approved, the Import/Export Coordinator will contact the shipping institution/animal source and advise them that DCM is prepared to receive the animals. Shipping arrangements are made by the shipping institution/animal source.

Step 4 – The Import/Export Coordinator notifies the investigator and lab contact person, the relevant DCM Area Supervisor and DCM's Veterinary Technical Services Manager of the animals' delivery date. Gift animals can only be received Monday-Thursday, 8 am-1:30 pm.

INVESTIGATORS MAY NOT AUTHORIZE SHIPMENTS OF ANIMALS: Unauthorized shipment of animals will result in euthanasia upon arrival or in being returned to sender, at

DCM's discretion.

Embryo Transfer Rederivation for Mice and Rats

All mice or rats imported from non-approved vendors typically undergo embryo transfer rederivation at MIT in order to minimize infectious disease outbreaks. Investigators need to complete the "Embryo Transfer Request and Billing" form (contact the Import/Export, Coordinator, animal-import-export@mit.edu) and will be billed for the cost of rederivation.

If incoming mice or rats from non-approved vendors are to be used only for tissue harvest or short pilot studies, there are quarantine housing options in 56, and rederivation may not be required. Contact DCM's Attending Veterinarian and Animal Resources Manager for more information. DCM approval is required to bypass rederivation and allow animals to be housed in 56 under quarantine

Obtaining Post-mortem Tissues

Obtaining post-mortem tissues from vendors or from extra animals euthanized by DCM or by another laboratory requires advance CAC approval. Submission and approval of a Tissue Harvest Exemption protocol must be in place prior to harvesting or utilizing tissues.

Any tissues from outside sources / vendors, including cell lines, should not be implanted or injected into MIT's animals due to pathogenic concerns unless explicit permission and/or testing for adventitious pathogens occurs under DCM's guidance.

Commercial Products and Contracting Offsite Animal Procedures

Animal-derived laboratory reagents (e.g., cells, antibodies) that are "mass produced" by a vendor, listed in a catalog, and available "as is" to the entire academic/research community, do not require any CAC or DCM authorization to procure. This is because the vendor is producing the product routinely. If the product is produced specifically for the buyer or customized CAC approval is required.

With an approved CAC protocol in place, a "Request for Laboratory Animal Purchase and Care" form needs to be filed with DCM in advance of each production/performance episode; this form can be provided by DCM's Animal Purchasing Agent, animalrequests@mit.edu. The vendor and species listed on the form need to match those described in the approved protocol, and the quantity entered on the form should include only the number of animals that are just about to be used. A new form needs to be submitted to DCM for every production episode that occurs throughout the lifetime of the protocol so DCM can track ongoing animal usage under the protocol. It is the investigator's responsibility to prepare the purchase order requisition (institutional purchasing cards should not be used), and we expect MIT's Sourcing and Procurement office to confirm with DCM that the paperwork was submitted before the purchase order is authorized and the production proceeds.

Quarantine

The purpose of quarantine procedures for newly arrived animals is to minimize the possibility of disease transmission and to ensure that animals are in optimal condition before an experiment. The duration of quarantine depends on the species, the source of the animal, and the intended use of the animal. This may vary by species and is often directed by DCM's Attending Veterinarian in coordination with the Clinical Veterinarian overseeing the facility and/or species. General guidelines are indicated below. The investigator is responsible for all diagnostic expenses associated with the quarantine of a particular species. While animals are in quarantine, they are the responsibility of the DCM veterinary staff. Investigators will not be consulted for routine treatments and are not permitted to use animals in quarantine without special approval of the veterinary staff.

Animal Stabilization and Acclimation Prior to Use

After receipt, all animals must undergo a stabilization and acclimation period of a minimum of 72 hours, unless express written permission is obtained from the Attending Veterinarian and/or a shorter acclimation period must be scientifically justified and specifically approved in the CAC protocol prior to animal arrival.

This acclimation period is to allow animals to adapt to their new environment, recover from the stress of shipping, allow DCM's veterinary staff to observe animals for any abnormalities not seen upon arrival and to condition animals to ensure that they are the best research subjects.

Note: nonhuman primates and some zebra finch imports must undergo extended periods of acclimation and testing (i.e. a minimum of 6 weeks) as guided by DCM's SOPs and/or at the direction of the Attending Veterinarian.

Animal Transfers - Rodents

Transfer of Animals within DCM MIT/WIBR Facilities

The facility veterinarian must approve the transfer of rodents between rooms or facilities on MIT campus and WI. A "Request for Rodent Relocation" form found on the DCM website must be completed. Investigators must also place DCM Animal Transfer cage cards on the cages to be transferred. DCM staff will move the animals and adjust census records.

Transfer of Animals from One Protocol to Another on Campus

When an investigator would like to transfer rodents to a different protocol (whether their own or another PI's) or animal room at MIT/WIBR they must complete an online "Request for Rodent Relocation" form. DCM staff will move the animals and adjust census records.

If the specific animal strain to be received is not already included on the protocol, the

receiving lab is required to submit a CAC Supplement F addendum and cover letter signed by the PI. The addendum must be approved by the CAC before the transfer can occur. Contact the CAC at cacpo@mit.edu.

Contact the DCM facility veterinarian and the CAC Office for guidance on transferring non-rodent species.

Transfer of Animals to Other Institutions (Domestic or International)

Note that any vehicle used to transport animals must be climate-controlled and approved by the CAC; public modes of transportation (such as subway or taxi) must never be used for the transport of research animals.

The welfare of animals being transported is of critical concern to the biomedical research community and constitutes one of our many obligations to ensure the safety and good health of laboratory animals. Before shipping animals to other institutions in a permanent transfer, DCM's Import/Export Coordinator (animal-import-export@mit.edu) must be contacted.

DCM uses CAC-approved courier companies for the transportation of laboratory animals. Shipping of animals can be delayed depending on weather conditions. As there is no consistent policy among courier companies, DCM has established temperature regulations under which animal shipments may occur, e.g., rodents may be shipped when outdoor temperatures are between 45-75° F.

Investigators wishing to ship rodents when temperatures are between 35-45° F or 75-85° F must obtain the permission of a DCM veterinarian. The investigator and veterinarian must sign a "Letter of Acclimation" (obtained from the Import/Export Coordinator), authorizing the shipment. For species other than rodents, DCM will exercise its judgment in allowing shipment during temperature extremes.

PROCEDURE FOR HEALTH CERTIFICATE AND SHIPPING ANIMALS TO OUTSIDE INSTITUTIONS

When shipping animals to outside institutions, the investigator must contact DCM for a veterinary health certificate and health report. The Import/Export Coordinator sends the health certificate and health report to the receiving institution, makes shipping arrangements with a CAC-approved courier company, arranges for a DCM veterinary technician to perform a final health check, arranges for the animals to be packed by a DCM Animal Technician, and provides shipping container(s).

Step 1 – Submit a "Request for Health Certificate and Shipping Animals" form to the Import/Export Coordinator.

**If the receiving institution is covering the costs associated with the shipment, a hard copy of a Purchase Order from the receiving institution's Sourcing and Procurement office must be submitted along with the*

request.

**If the MIT/WIBR investigator is covering the costs associated with the shipment, an account number for billing must be listed on the "Request for Health Certificate and Shipping Animals" form.*

The Purchase Order should be made out to: MIT- DCM
77 Massachusetts Avenue
Building 16-849
Cambridge, MA 02139

Step 2 – The Import/Export Coordinator will send a veterinary health certificate and health report to the receiving institution.

Step 3 - The Import/Export Coordinator must wait for approval from the receiving institution veterinarian before making shipping arrangements. **The lab contact person must place DCM lavender cage cards, reading "Animals to be shipped out", on the cage(s) of animals to be shipped.**

Step 4 – Once DCM has approval to ship from the receiving institution veterinarian and a copy of the Purchase Order (if needed), shipping arrangements are made by the Import/Export Coordinator. The latter notifies MIT/WIBR investigator and the receiving institution of the shipping date and arranges for a DCM veterinary technician to perform a health check and an Animal Technician to pack the animals. An approved shipping container(s) is also provided.

Animal Transfers – Other Species

Contact the DCM facility veterinarian and the CAC Office for guidance on transferring non-rodent species. In general, naïve animals which are USDA-covered species may be transferred from one protocol to another with direct approval by the Attending Veterinarian, or their designee and the CAC. A transfer form must be submitted to cacpo@mit.edu. This transfer form details the history of the animal, any experimental manipulations and suitability for transfer. For those animals in which a surgical procedure has been performed, a request for transfer may need to be sent to the USDA/APHIS by the IO, in writing, and receive approval before an animal may undergo multiple major survival surgical procedures in separate research protocols.

Animal Adoption Policy

Animal adoption as a means of disposition of research animals will be considered on a case-by-case basis by the CAC upon the recommendation of the DCM veterinary staff that the animal(s) involved may be suitable as pets. Adoption of genetically engineered (i.e., transgenic) animals or animals that have been subjected to major procedures having the potential to affect the behavior, physiology or suitability of the animal as a pet will not be considered. Animals that are considered for adoption must meet behavioral and health criteria judged by the veterinary staff, and then will be offered for adoption without a guarantee of health, behavioral suitability as a pet, or absence of zoonotic disease. Investigators are reminded that research animals are the property of MIT/WIBR and their disposition after use must follow MIT/WIBR guidelines.

Chapter 5: Animal Use

Animal Identification

Printed Cage Cards, Tags, Tattoos, Other

- **Printed cage cards are utilizied for all mammals except macaques and marmosets.** Animals must be identified by a cage card secured to the cage. Animals purchased through DCM will be accompanied by printed cage cards listing PI, CAC protocol number, account number, and contact person information (including name, lab extension, email address, cell phone number). Investigators are responsible for the placement of additional cage cards on cages with animals generated by weaning or cage separations, and new cage cards must list the same information as given above. Cage cards can be ordered free of charge using the Rodent Cage Card Order Form on the DCM website.

- Rodents. Individual identification of rodents can be performed using ear punch/notches, ear tags, tattoos and implantable microchips, none of which require anesthesia. If ear tags are used, they should be placed at the 4 o'clock position on the ear so the tag does not fold the ear over or hang down against the neck, predisposing the animal to ear trauma or infection. Methods used to identify rodents should be clearly described in your CAC protocol and be approved by the CAC.

- Rabbits. Vendors tattoo an identifying number in the ear. This number should be written on the printed cage card where the rabbit is housed.

- Ferrets and swine. These come with identifying numbers from the vendor (such as ear tags or tattoos).

- Nonhuman primates. Nonhuman primates are given an identification number/DCM tattoo during quarantine, and these numbers are used in all documentation pertaining to the animal. A plastic tag with the DCM identification number is usually affixed to the animal's cage. For common marmosets, cage cards can be used, and when animals are >6 mo or >300 grams, they are provided a permanent tattoo.

- Amphibians and reptiles: Each tank/enclosure should have a printed cage card.

- Zebrafish: Each individual tank should be labeled appropriately with protocol number, date of birth (if known), species, genotype (if applicable/known). If the written text becomes faded, the label should be rewritten or replaced. Laboratory contact information should be easily discerned by either directly from the label or by CAC protocol posted in animal holding room location.

- Zebra finches. Contact information sheets with PI, protocol number, contact name, email, and cell phone number are posted on cubicle/room doors. Leg bands are acceptable for identifying individual birds; these must be described in an approved CAC protocol

Toe-Clipping for Rodent Identification

According to the Guide, toe clipping should only be used as a method of identification for small rodents when no other individual identification method is feasible. See CAC Policy on Toe-Clipping for further detail. Toe clipping requires scientific justification to the CAC that no other method is suitable, and is limited to pups up to 7 days of age. Additional training may be required.

Behavior Testing Considerations

General comments:

For all behavioral testing and tests with the potential for pain and distress, proposed procedures for monitoring, record keeping, and humane intervention and endpoints should be described in the animal-use protocol and approved by the CAC. All studies will be in accordance with governmental regulations, including recommendations in the Guide for the Care and Use of Laboratory Animals (ILAR, National Resource Council, 2011) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (ILAR, National Resource Council, 2003)

A baseline behavioral profile of an animal should be attained if changes in behavior are going to be used to monitor for distress. If such changes are observed, the researcher should contact DCM veterinary staff so that the animal can be assessed further. Any experiments deviating from the general guidelines below must be justified based on scientific objectives and approved by the CAC.

Behavior Test Design

Behavior testing is common for many types of research, particularly in the field of neuroscience. When designing a behavioral test, the following points should be considered:

- The severity and duration of tests should be minimized. The order in which tests are performed should also be thought out beforehand, often starting with the least stressful or invasive test is recommended.

- As different strains and species of animals apportion activity between light and dark periods differently, and levels of expression of genes can change with time of day, the time that tests are conducted needs to be carefully selected.

- The perceptual and behavioral characteristics of the animals to be tested, which may be affected by age and experience, should be considered.

- Housing enrichment is standard for all animals in DCM facilities. Group housing is strongly encouraged as many species benefit from social interaction. If single housing is required as part of the experiment, scientific justification must be provided to and approved by the CAC. All singly housed rodents require a “Single Housing” cage card

and additional enrichment.Guidelines for Rodent Behavioral Tests

- Behavior testing must be conducted in lab spaces that have been approved by the CAC and DCM veterinary staff. DCM staff will routinely visit rodent behavioral testing areas to assure orderly and sanitary conditions and practices. Guidance for Behavioral Testing Spaces are outlined in the CAC Policy Behavioral Testing Areas and Equipment.

- Overlap of different species and/or health status of animals within behavioral testing areas should be minimized whenever possible.

- Appropriate PPE (personal protective equipment) must be worn by researchers while behavioral testing is performed. This includes a hair bonnet, lab coat, mask and gloves when handling the animals.

- All equipment in contact with animals must be able to be sanitized. Equipment should be taken apart and thoroughly cleaned on a regular basis. Equipment holding food and water should be cleaned at least once a week, or more often depending on the frequency of use. In particular, no cardboard, styrofoam, exposed wood or tape should be used in behavior test setups.

- Extraneous clutter should be kept to a minimum. Trash should be disposed of properly and barrels emptied as needed. Floors should be swept and mopped as needed to keep them free of feces, bedding/nestlet materials, and other debris.

- If an animal becomes compromised, injured or ill while in the behavior testing spaces, DCM veterinary staff and/or the Area Supervisor must be contacted immediately.

Prior to Starting a Behavior Test

- Stopping points for behavioral testing should be task specific, e.g., when an animal shows signs of defeat, rather than being of a fixed duration. When studying social defeat, appropriate intervention or barriers and escape routes should be provided whenever possible.

- When possible, acclimation to the test, limits of attempts, and a finite duration of the behavior test (ex: Water Maze) must be described in the CAC protocol.

- Every animal room has a regular cage changing schedule. Animals which undergo behavioral testing daily, or on a frequent basis, may require special arrangements to have their cages changed. Note that cage changing may change the behavior of the animal, thus, familiarization with the schedule is beneficial for planning a testing schedule. Such arrangements need to be made with the Area Supervisor.

- Investigators are encouraged to spend a few days prior to the start of experiments handling their animals. This will help the animal acclimate to the handler, keeping in mind variables such as color of lab coat and gloves, scents and sounds; potential stress

and a learned aversion to handling can be reduced in this way

- **Animals must be signed in and out of the facility, and cages must be completely covered with an opaque covering upon leaving the facility.** Water bottles in rodent cages must be removed or flipped during transfers, and animals returned to water access upon arrival.

- Animals should spend minimal time outside of the animal facility, and no animals may be housed overnight in laboratory spaces unless prior approval has been given by the CAC.

- Leaving the animals in the testing space for 30-60 minutes prior to the start of experiments will allow them to acclimate to the environment. Appropriate lighting and sounds in the testing spaces need to be considered.

- Within the facility, "Experiment in Progress" cards can be used to convey the type, description, duration and number of cages on experiment. This is especially important for experiments that are being conducted within the animal facility itself. **Contact information must be provided, including a cell phone number, to ensure prompt response and care for the animals.**

During a Behavior Test

- The experimenter is the largest single influence on test scores. Proper randomization and balance of the test order is usually required. Also keep in mind that, as with handling, associations with colors and scents may have a profound effect on the response of the animal.

- All equipment must be sanitized between animals. This is to reduce the effect of odors, pathogen transfer, and to ensure that each testing space is consistent between animals. For example, wet surfaces like urine on a fear conditioning grid can alter the resistance.

- Behavior testing often overlaps with other common procedures such as surgery and food/water regulation. Ensuring good surgical technique and postoperative care allows the animals to recover optimally and reduces the time before behavior testing can commence. It also reduces the chances of subclinical infections and the loss of animals due to, for example, implant failure. Food or water regulation is also conducted to improve motivation. The time when food/water is provided is critical to ensure that hunger or dehydration does not interfere with the learning of tasks. **If possible, more palatable foods should be used in place of food and water regulation.**

Food and Water Regulation

Rodents used for experimental purposes at MIT/WIBR routinely receive ad libitum facility water and a standardized DCM-approved diet formulation that supports proper nutrition and reproducible research data. Any exceptions to this dietary protocol require CAC approval.

Affiliate with the "Req for: Rodent Food & Water Regulation" Training Group in the MIT Learning Center and complete the associated training requirements. CAC approved guidelines can be found in the "Diet Modifications" policy on the CAC website.

No animal can be food or fluid restricted without CAC approval. Both food and water cannot be restricted at the same time.

Assigning Pain and Distress Categories to Animals Involved in Behavioral Testing:

Category	Description	Examples
C	Procedures which do not involve pain and/or distress, involve only very slight and momentary pain (e.g. needle stick), and for which the use of anesthetics, analgesics or tranquilizers are not indicated or required.	*Non-invasive and "low impact" studies:* activity and observation, open-field, mazes and other tests that allow animals to explore and move to preferred locations, conditioned place preference; positive reward conditioning; Allodynia tests (e.g., von Frey, hot plate, tail flick), gait analysis, strength and climbing tests, rotarod and balance beams; object recognition, marble burying; running wheel; food/fluid preference
D	Procedures that would be expected to cause more than slight or momentary pain or distress that is appropriately alleviated by anesthetics, analgesics, tranquilizers, removal from study, euthanasia, or other approved methods. This category implies that the "pain" is in excess of that caused by injections or other minor procedures (USDA Policy #11).	*If pain, agitation and/or distress are noted, then animals are at least removed from the test/study, and treated (if applicable):* -Forced aggression (e.g., intruder, social defeat) -Treadmill and forced exercise -Morris water maze -Fear conditioning (foot shock) -Self-administration and extinction of drugs in addiction models -Novelty-induced hypophagia -Variable stress (constant bedding changes, cage manipulations, exposure to predator scents, absence or abundance of enrichments)
E	Procedures that would be expected to cause more than slight or momentary pain or distress NOT alleviated by anesthetics, analgesics, tranquilizers or other approved methods, i.e. pain in excess of that caused by injections or other minor procedures (USDA Policy #11).	*Behavioral tests that require inducing pain or distress:* -Sleep deprivation (>24 hours) -Depression tests (e.g., forced swim test, tail suspension test, learned helplessness) -Inescapable foot shock -Untreated withdrawal from induced addiction -Maternal separation -Chronic unpredictable stress (CUS) model (when used as a model of depression)

Guidance on Rodent Behavioral Tests:

Tail Suspension Test (TST)

- **Purpose:** This test is used to assess depressive-like behavior (or a form of learned helplessness) and is useful in the screening of potential antidepressant drugs. In the TST, a mouse is suspended by the tail, in a position where it cannot escape or hold on to nearby surfaces. After initially attempting escape by engaging in vigorous movements, mice rapidly become immobile. Longer periods of immobility are associated with a more depressive state.
- **Species used**: Mice, rats
- **USDA Category**: E
- **Recommendations:**
    1. For suspension of the mouse, vinyl or medical adhesive tape is recommended. Duct tape is too adhesive and can tear hair and skin when removed[1]. **The tape should be applied in a consistent position ¾ of the distance from the base of the mouse's tail**[3]. If the tape is applied too near the tip of the tail it may pull off the skin of the tail tip and the mouse will fall.
    2. Care should be taken to ensure there are no differences between groups in motor function, motor coordination, endurance, and balance.
    3. The test performer should be **present and observant** throughout the duration of the procedure. Should an animal show undue stress, e.g., excessive vocalization, tail damage from struggling, the mouse should be removed from the apparatus.
    4. Some strains (e.g., C57BL/6) may not perform well in the TST due to **tail climbing behavior**. Strains with vestibular deficits may show an abnormal spinning phenotype and should not be used in the TST. Other mouse phenotypes that display neurological abnormalities that lead to unusual leg clasping behavior or that influence immobility times may also not be appropriate models for this test[1].
    5. The test for each mouse should **not last for more than six minutes**, and repeated no more than **four times** in one session[1]
- **Alternative tests**: Porsolt swim test, others
- **References:**

    1. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. 2012. The Tail Suspension Test. J Vis Exp 59:3769.
    2. CL Bergner, AN Smolinsky, PC Hart, BD Dufour, RJ Egan, JL LaPorte, AV Kalueff. 2010. Mouse Models for Studying Depression-Like States and Antidepressant Drugs. In: Mouse Models for Drug Discovery, Methods in Molecular Biology 602: 267-282.
    3. Castagné V, Moser P, Porsolt RD. Behavioral Assessment of Antidepressant Activity in Rodents. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press; 2009. Chapter 6.
    4. B Thierry, L Steru, P Simon, RD Porsolt. 1986. The tail suspension test: Ethical considerations. *Psychopharmacology* 90: 284-285.

Forced Swim Test (FST)

- **Synonyms:** Porsolt swim test, behavioral despair test
- **Purpose**: This task is used to assess depressive-like behavior (or a form of learned helplessness). In the FST, the animal is placed in a circular tank of water from which it cannot escape. Animals that are "depressed" will commonly stop swimming and float at the water surface quickly, while "non-depressed" animals continue to swim in an attempt to find the platform.
- **Species used**: Mice, rats
- USDA Category: E
- **Recommendations:**
    1. Water depth and temperature
        a. The water depth should be enough so the animal cannot touch the bottom with its tail or feet. A depth of 30 cm is commonly recommended, and at least 15 cm from the top to avoid escape from the top of the container[2].
        b. The water temperature should be 24-30°C.
        c. **After completion of the trial,** the animal should be dried using an absorbent towel and placed back in its home cage (which has been warmed with a warm water blanket or hand warmers under half of the cage). Heat lamps or a blow dryer can be used but caution must be taken to not overheat the animal or cage environment. Take care in hand drying animals with a paper towel as the animals may find this stressful and rough handling can cause injury.
    2. Test procedures
        a. Animals subjected to the test must be in good general health, and observed continuously during the swim test. Any animal that fails to swim or float (sinks below the water surface) should be removed from the water immediately[2].
        b. Impaired swimming ability due to musculoskeletal or other abnormalities will affect performance in this test.
        c. Care should be taken to ensure no differences between groups in motor function, coordination, and balance.
        d. The test session duration should **last no longer than 15 minutes**[1].
        e. Ensure that the animals are recovered (ambulating normally, grooming, eating) and not exhibiting signs of distress (walking abnormally, hunched, not moving, increased respiratory rate) before returning them to the animal holding room.
    3. Cleaning and sanitization
        a. Tanks must be drained and cleaned after each day's trials.
        b. Any accumulating debris (e.g., feces, bedding) should be picked up and discarded using a soft mesh net that is easily sanitizable.
- **Alternative tests**: Tail suspension test, others[1,2,3].
- **References:**

1. Crawley JN. 2007. What's Wrong With My Mouse? Behavioral Phenotyping of

Transgenic and Knockout Mice, 2nd ed. Hoboken (NJ): Wiley.
2. *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. 2003.Washington, DC: The National Academies Press. Available from: **http://www.ncbi.nlm.nih.gov/books/NBK43327/**
3. Castagne V, Moser P, Porsolt RD. 2009. Behavioral Assessment of Antidepressant Activity in Rodents. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press. Chapter 6. Available from: **http://www.ncbi.nlm.nih.gov/books/NBK5222/**

Morris Water Maze (MWM)

- **Synonyms:** Morris water escape or navigation task, submerged platform water escape task
- **Purpose:** This test is used to evaluate spatial learning and memory. It takes advantage of the natural swimming ability of rodents and their motivation to escape from the water. Spatial orienting cues are placed around the arena and mice learn the location of an escape platform based upon those cues. Additives to make the water opaque, such as non-toxic white tempera paint, are used so the animal is forced to rely on external/extra-maze cues, and not see the platform, nor rely on olfactory cues, to find the escape route.
- **Species used**: Mice, rats
- **USDA Category**: D
- **Recommendations**:
    1. Relevant controls: **In mice, performance in this test is heavily dependent on genetic background**. Special consideration must be given to the use of this test in mouse strains or genotypes with reduced ability to navigate using spatial cues or to swim, e.g., visual or musculoskeletal impairments. For example, FVB/N and C3H/HeJ mice have significant vision impairment due to retinal degeneration. In addition, mice with other physiological or behavioral traits such as impaired thermogenesis or high anxiety levels may also perform poorly in this test.
    2. Water depth and temperature:
        a. The water temperature should be 22-28°C, and recorded for each test period in a notebook that is available at all times for review by veterinary staff, CAC members and regulatory inspectors.
        b. **Water temperatures < 20°C can lead to hypothermia**. Temperatures that are too warm may discourage active swimming/searching.
        c. The risk of hypothermia should be lessened by increasing the inter-trial interval/recovery time.
        d. Water depth should be no more than 60 cm. (~two feet). The tank should be filled so the platform is one inch below the surface of the water.
        e. **After completion of the trial,** the animal should be dried using an absorbent towel and placed back in its home cage (which has been warmed with a warm water blanket or hand warmers under half of the cage). Heat lamps or a blow dryer can be used but caution must be taken to not overheat the animal or cage environment. Take care in hand drying animals with a paper towel as the animals may find this stressful and rough handling can cause injury.

f. Ensure that the animals are recovered (ambulating normally, grooming, eating) and not exhibiting signs of distress (walking abnormally, hunched, not moving, increased respiratory rate) before returning them to the animal holding room.

3. Test Procedures
    a. Trials should not exceed two minutes.
    b. Inter-trial intervals should be long enough to prevent the development of hypothermia and fatigue over repeated trials. **It is recommended that rest intervals be at least 10-15 minutes**, especially in mice[5].
    c. **Animals must be continuously monitored** while in the tank and removed from the water if their head sinks below the surface[4].
    d. Two to four trials per day are generally adequate for learning the task[5].
    e. Items used as spatial cues must be sanitizable or disposable.
    f. A water maze worksheet should be posted inside the room housing the water maze; individuals cleaning and re-filling the water maze should sign and date the sheet.
    g. DCM staff will routinely visit rodent behavioral testing areas to assure orderly and sanitary conditions and practices.
4. Cleaning and sanitization
    a. Tanks must be drained and cleaned once per week, or more often as needed: drain pool completely and remove all solid material, rinse with high pressure water, and scrub before refilling.
    b. Additives used to make the water opaque must be stated in the protocol, non- toxic, and easily sanitizable when the tank is drained and cleaned. Powdered milk decays rapidly and should not be used. Any paint spills should be mopped up immediately.
    c. Any accumulating debris (e.g., feces, bedding) should be picked up and discarded using a soft mesh net that is easily sanitizable.
    d. Refer to the CAC policy on 'Behavioral Testing Areas & Equipment' for more information.

- **Alternative tests for spatial learning and memory**: T-maze, Barnes circular platform maze, radial arm maze
- **References:**

1. Terry AV Jr. 2009. Spatial Navigation (Water Maze) Tasks. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press. Chapter 13. Available from: http://www.ncbi.nlm.nih.gov/books/NBK5217/
2. Crawley JN. 2007. What's Wrong with My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice, 2nd ed. Hoboken (NJ): Wiley.
3. Wahlsten D. 2011. Mouse Behavioral Testing: How to use Mice in Behavioral Neuroscience. Amsterdam: Elsevier.
4. *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. 2003. Washington, DC: The National Academies Press.
5. Iivonen H, Nurminen L, Harri M, Tanila H, Puolivali J. 2003. Hypothermia

in mice tested in Morris Water Maze. Behav Brain Res 141(2): 207-213.
6. Baker R. 2013. Water maze. Available from: http://www.watermaze.org.

Sucrose Preference Test (SPT)

Purpose: This test is used in a model of affective disorders, including depression, to indicate anhedonia (lack of interest in rewarding stimuli). The animal's interest is assessed in seeking out a sweet rewarding drink (sucrose solution) relative to drinking water. A bias towards the sucrose water is typical, and a failure to do so is indicative of anhedonia/depression.
Species: Mice, rats

USDA Category: C

Recommendations:

1. Prior to testing, rodents should be habituated to the presence of two drinking bottles for at least 24 hours in their home cage. This also serves to reduce neophobia to sucrose solution[1,2].
2. To prevent leakage, which can reduce undesired variability, monitor for any problems with liquid flow and observe for signs of spillage, *e.g.,* wet bedding below the sipper tube[1].
3. Whenever possible, any food or liquid rewards utilized in behavioral experiments should be supplied from vendors of rodent diet products or commercial products that are approved for use by DCM (e.g., Bio-Serv sucrose pellets, ClearH2O sucrose solution).
3. If sucrose solution is prepared in the lab, it should be prepared fresh and refrigerated, with a label of the concentration and date made. The solution should be discarded after 30 days. Exposing sucrose solution to room temperature for longer than three days may lead to growth of bacteria and fungus[1].
4. No previous food or water deprivation should be applied before the test.
5. Rodents can develop a bias for one side of the cage or the other, resulting in increased intake from one bottle. Thus, the bottle position for each cage should be switched daily to avoid false sucrose preferences resulting from side bias[1].

Alternative tests: Sweet Drive Test

References:

1. Eagle AL, Mazei-Robison M, Robison AJ. 2016. Sucrose Preference Test to Measure Stress-induced Anhedonia. Bio-protocol 6(11): e1822.
2. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC et al. 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131(2): 391-404.

3. Mateus-Pinheiro A, Patrício P, Alves ND, Machado-Santos AR, Morais M, Bessa JM, Sousa N, Pinto L. 2014. The Sweet Drive Test: refining phenotypic characterization of anhedonic behavior in rodents. Front

Behav Neurosci 8:74.

Platform or Arena Testing (e.g., Elevated Plus Maze, Zero Maze, Novel Object Recognition)

Recommendations:

1. All tests involving a platform or arena set-up (e.g., Elevated Plus Maze, Barnes Maze, T maze, Radial 8-Arm Maze, Novel Place Recognition, Novel Object Recognition) should have a sturdy, non-porous, easily sanitizable platform that is cleaned between animals with accelerated hydrogen peroxide disinfectant, ethanol, 0.5% acetic acid, or 10% dilute bleach. Refer to the CAC policy on 'Behavioral Testing Areas & Equipment' for more information.
2. During the test session, animals must be **continuously monitored**. The **criteria for intervention** and removal of the animal under each specific test must be stated in the experimental protocol and followed accordingly – e.g., prolonged immobility, increased respiration rate, lethargy.
3. Due to the height of many of the platforms, some form of sanitizable or disposable padding should be placed on the floor to prevent injury in the event an animal falls. Platforms and arenas must be no more than four feet in height.

Chronic unpredictable stress (CUS) model

- **Synonyms:** Chronic mild stress (CMS) model
- **Purpose:** Animals are subjected to various unpredictable stresses as a measurement of depression. The CUS model of depression relies on a series of mostly physical stresses that are presented over one to six weeks[1].
- **Species:** Mice, rats
- **USDA Category**: E
- **Guidelines:**
  1. **Veterinary staff must be notified** before starting the stressor(s) and testing period.
  2. For each stressor/test detailed in the animal-use protocol, provide the **time required** for each testing and/or training session, the **frequency** of testing/training sessions and **intervals** between sessions, and the **duration** of testing/training sessions. A table or graph may be used to help describe this in the protocol.
  3. Describe the **limits** to deprivation or aversive stimuli if the desired response does not occur, and criteria for intervention and removal from the stressor.
  4. Animals currently under the CUS model should be **monitored daily** to assess hydration, appetite, activity, and urine and fecal collection. Animals should be **weighed at least three times weekly** during the stress and testing period. If animals are decreasing in weight, daily weights should be taken.
  5. Stressor procedures should be carried out in isolated rooms adjacent to the housing room, requiring minimal handling or transport of the animals.
  6. Be aware that being exposed to intense, repeated, or prolonged stressors can cause adverse effects on the health of the animals, including suppression of reproduction, immune dysregulation, cardiovascular and gastrointestinal impairment, and disrupted

neuroendocrine function[2]. These can precipitate the development of abnormal behaviors, including self- mutilation, mutilation of other animals, and stereotypic behaviors (such as bar-chewing).

7. Recorded observations on health parameters of rodents (weight, behavior, appetite, activity level, hair coat), as well as a history of stressor/test timetable should be available at all times for review by veterinary staff, CAC members and regulatory inspectors.
8. After conducting each stressor, the animals should be monitored for at least 15 min in a cage with access to ad libitum food and water. Ensure that the animals are recovered (ambulating normally, grooming, eating) and not exhibiting signs of distress (walking abnormally, hunched, not moving, increased respiratory rate) before returning them to the animal holding room.
9. If at any point there is a decline in health as stipulated by the humane endpoints dictated in the protocol, the animal should be removed from the study. Animals displaying signs of unmanageable pain or distress should be euthanized, even if prior to the experimental endpoint. DCM veterinary staff should be contacted to help assess the animals' general health and wellbeing.

Guidance on specific stressors:

Note: Due to the many variable stressors included in this model, these guidelines are not exhaustive. We recommend consultation with DCM veterinary staff for review and guidance if planning to start this model for the first time.

1. *Soiled bedding:* This stressor should be standardized in some way (e.g., metabolic cage, collect and measure amount of urine and defecation, then combine this with fresh mouse bedding).
2. *Tail pinching:* This stressor should be applied no longer than 60 seconds, approximately 1 cm from the end of the tail with a clothespin or similar. Be sure to add monitoring for morbidity of the tail site (e.g., skin sloughing, dark-colored or necrotic tail) and criteria for removal from this stressor.
3. *Cold or Warm Swim:* The duration of this stressor should be no longer than 5 min. Animals should be continuously monitored during the application of this stressor. Please note the criteria for intervention (e.g., head goes and stays under water). Include means for temperature monitoring before each stressor, and sanitization of the tank after a daily trial period. Add information about post-swim monitoring, drying of the animal, and recovery. *See other swim test guidelines for recommendations on monitoring, sanitization etc.*
4. *Prolonged restraint:* Animals should not be restrained for more than two hours, unless scientifically justified. Minimize exposure to urine and feces soiling of the animal under the restraint stressor. Restraint devices should be cleaned between rodents with accelerated hydrogen peroxide disinfectant, ethanol, or 10% dilute bleach. Please see the CAC Policy on 'Physical Restraint' for more information.
5. *Social Defeat:* Be sure to include explicit criteria for intervention, session duration, and frequency and duration of monitoring.

6. *Food Regulation / Deprivation (24 hr):* This stressor should not be administered right after a period of water deprivation; please refer to the CAC policy on 'Diet Modification' for more information.
7. *Water Regulation / Deprivation (24 hr):* This stressor should not be administered right after a period of food deprivation; please refer to the CAC policy on 'Diet **Modification' for more information.**

References:

1. Willner P. 2005. Chronic mild stress (CMS) revisited: Consistency and behavioural- neurobiological concordance in the effects of CMS. Neuropsychobiology. Karger Publishers.
2. Moberg GP, Mench JA. 2000. The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Wallingford, Oxon, U.K.: CAB International.
3. Zimprich A, GarrettL, et al. 2014. A robust and reliable non-invasive test for stress responsivity in mice. Front Behav Neurosci. 8: 125.

Fear Conditioning (with use of foot shock)

Purpose: Pavlovian fear conditioning (FC) has been well used to illustrate associative learning in many species, most commonly in mice and rats[1]. The dependent measure used in contextual and cued (delay or trace) fear conditioning is a post-exposure freeze response that takes place following pairing of an unconditioned stimulus (US), such as a foot shock or air puff, with a conditioned stimulus (CS), a particular context and/or such a cue. In the case of rats and mice, this US is generally a mild foot shock.

- **Species used**: Mice, rats
  - **USDA Category**: D; if inescapable, E
  - **Recommendations:**
    1. The test chamber should be constructed to be easily cleaned and have a way to easily view the animal being tested.
    2. Before starting, the chamber should be wiped with accelerated hydrogen peroxide disinfectant, ethanol, or 10% dilute bleach. Between animals in testing, the chamber should be cleaned/wiped out with the same disinfectant. This will minimize olfactory cues from other animals tested previously.
    3. Considerations for FC using foot shock, adapted from Curz P, et al.[2]: The level of shock produced as the US will vary depending on the shock source and manufacturer. It is recommended to actually observe the animal and gradually increase the shock to a level where the animal first responds and rapidly runs around the cage. This level is lower than that where the animals start to jump to avoid the shock, although some strains may show a hyperresponsivity to an initial shock.
    4. Animals must be continuously monitored while applying the fear conditioning stimulus and test.
    5. Ensure recovery of the animal after foot shock paradigm. DCM veterinary staff should be consulted if there is any animal found with persistent increased respiratory rate, hunched posture, reduced activity, or other abnormal behavior after testing.

  References:

1. Kim JJ, Jung MW. 2006. Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. Neurosci. Biobehav. Rev 30(2):188-202.
2. Curz P, Rustay NR, Browman KE. 2009. Cued and Contextual Fear Conditioning for Rodents. In: Buccafusco JJ, editor. Methods of Behavioral Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC press. 1–12. Chapter 2. Available from: https://www.ncbi.nlm.nih.gov/books/NBK5223/

Blood Collection in Mice and Rats

General Guidelines for Blood Collection in Mice and Rats

Some of these methods may be adapted for other rodent species. Please discuss with DCM's veterinary staff.

Proper restraint or anesthesia of the animal is required for all methods of blood collection.

Training in these techniques is available through DCM; contact dcmtraining@mit.edu.

Aseptic technique is recommended for all blood collection methods, though terminal procedures may be an exception. The fur of the animal may need to be shaved or at least clipped from the site for better visibility, where needed. This is not indicated for retroorbital, submandibular or tail vein access). The area may be swabbed with alcohol and allowed to dry, although the smell of alcohol may be stressful to the animal. Alcohol decreases surface tension and results in dispersal of blood into the surrounding area, making collection difficult with some methods.

- In order to better visualize veins, dilation of vessels is often performed prior to bleeding. Adequate dilation of vessels can be achieved by the use of heat (expose animal to 37° C for 5-8 minutes or warm the area locally), or by proper restraint and occlusion of the vessel. Use of topical irritants is to be avoided.

- Needles of appropriate gauge must be selected for venipuncture procedures. For mice and rats, 23-to-30-gauge needles are used depending on the sampling site and animal size.

- Often proper insertion of the needle into a vein is the most difficult part of the procedure. Guidelines can be given but only practice will lead to proficiency. Vessels may be expected to roll, collapse or shift, making insertion difficult. A precise, careful introduction of the needle is always best, but several attempts may be required. The needle should be inserted parallel to the vein and the tip directed into the lumen along the longitudinal axis. When withdrawing blood from a vein, aspiration should be slow so the vessel is not collapsed. Stabilization of the syringe and needle is also critical during blood collection.

- Proper hemostasis is important to avoid excess bleeding, bruising, and/or hematoma formation. After blood collection, light pressure should be applied until bleeding stops. Animals should be monitored for several minutes once in their home cage to ensure

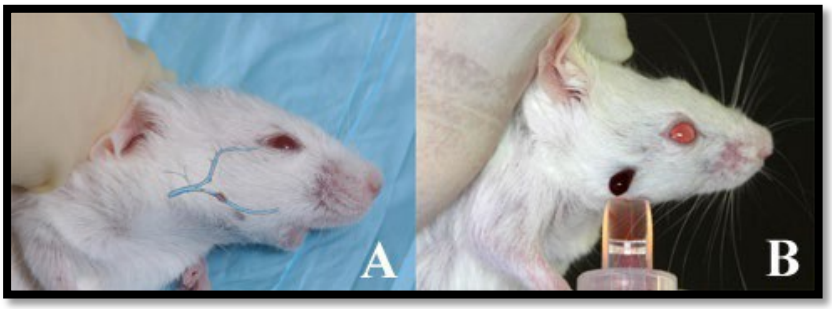
bleeding does not start again. Any signs of trauma to the area preclude that site from being accessed again until complete healing has occurred.

- The amount of blood that can be taken is an important consideration. **The maximum withdrawal volume allowed as a single event or over a period of two weeks is ~1% of the animal's body weight (i.e., 15% of the animal's total blood volume, where total blood volume (ml) is ~7% of the animal's body weight (g); refer to the table below)**

- The frequency of collection depends on the aims of the study and also on the maximum allowed volume over a two-week period. This must be defined in your CAC protocol. It is important to perform the minimum number of needle punctures and rotate sites to allow adequate tissue healing. Note: Blood sampled from different sites can result in different clinical pathology values.

Recommended Methods for Blood Sampling in Mice and Rats

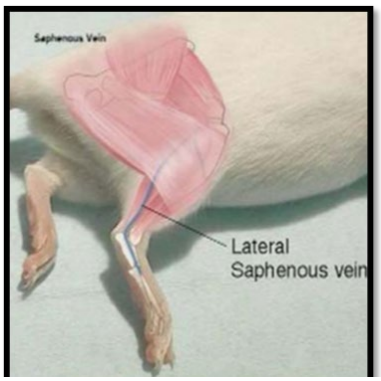
The submandibular vascular bundle technique (aka "cheek bleed") is a reliable, rapid method for serial blood sampling from mice, and does not require the use of anesthesia. The cheek pouch tissues are unaffected when the procedure is done correctly by trained personnel. The technique involves puncture of the submandibular vascular bundle, midway between the base of the ear and the ventral mandible using a disposable, sterile single-use mouse bleeding lancet (scalpel blades or needles may **not** be used). In general, it is at the level or the area of the "freckle" of the cheek of the mouse. Blood flow is terminated by gentle compression at the bleeding site. The submandibular vascular bundle is not visible during this procedure; therefore, the researcher should become familiar with the anatomy using a mouse cadaver prior to attempting this method on a live animal.



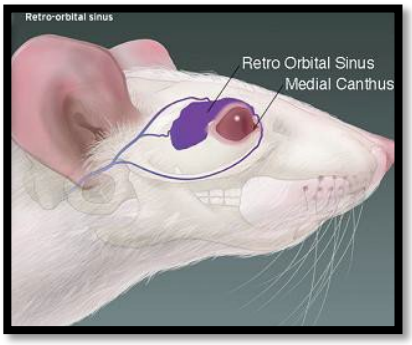
The lateral tail vein technique can be used in mice and rats for serial sampling without anesthesia, but a restrainer is required. A 27–30-gauge needle and syringe or lancet/larger- sized needle is used to make a laceration or puncture in the vessel found on either side of the tail, and blood is collected into a microhematocrit tube. This method is facilitated by warming the rodent using a heat blanket or heat lamp (with extremely close observation), or by warm water immersion of the tail to cause vessel dilation. The blood collected should flow freely, and "milking" the tail should be avoided.

The saphenous/lateral tarsal vein technique can be used in rats and mice; it does not require anesthesia and allows for repeated blood sampling. The saphenous vein is on the lateral aspect of the ankle (tarsal joint) and is easier to see when the fur is shaved, and the area wiped with

alcohol. The animal is placed in a suitable restrainer and the operator extends the hind leg. Petroleum-based ophthalmic ointment can be applied to prevent wicking of the blood into the surrounding area and make the blood to pool on the skin after venipuncture. The vein is raised by gentle pressure above the joint and is punctured using the smallest gauge needle that enables sufficiently rapid blood withdrawal without hemolysis, e.g., a 25-27 gauge needle. For small volumes, a simple puncture of the vessel with a needle leads to a drop of blood forming immediately at the puncture site; then a microhematocrit tube can be used to collect a standard volume. After blood has been collected, pressure over the site is sufficient to stop further bleeding. Removal of the scab will enable serial sampling.



The retroorbital sinus technique is reliable and relatively safe for serial blood sampling in mice and will give reasonable sample volumes; it is discouraged in rats. In mice, a comparable amount of blood can be collected via the submandibular or submental route, which may be preferred. Proper training and practice are required to prevent trauma to the eye. **This technique must be performed under general anesthesia**. It's encouraged to apply topical ophthalmic anesthetic agent to the eye before performing this technique. Slight thumb pressure is applied on the jugular vein to occlude venous return from the retroorbital sinus, followed by penetration of the retroorbital sinus (at medial canthus of the eye) with a sterile microcapillary tube, in one direction as the tube is advanced gently. Blood flow is terminated by release of pressure to the jugular vein and removal of the capillary tube. Pressure should be applied to the closed eye, following blood collection, to assist hemostasis and prevent hematoma formation.



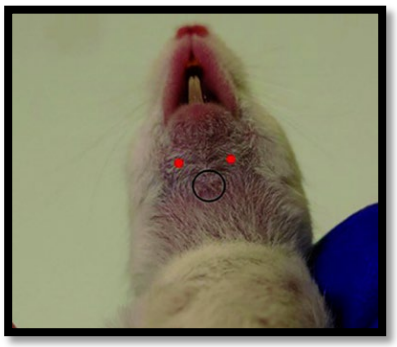


Dorsal pedal vein technique is similar to the saphenous technique and has been described in mice and rats. After adequate restraint, the distal limb is grasped at the tarsal joint and the pedal vein visualized. A 25–30-gauge needle is used to puncture the vein and blood is collected via capillary action into a microhematocrit tube. Serial sampling is possible by puncturing the vessel again at a different site or by removing a formed scab.



The jugular vein technique can be performed in mice and rats but requires anesthesia. With the area of the neck shaved and held in hyperextension, the jugular vein can sometimes be visualized just lateral to the sternoclavicular junction (arrow in diagram below). A 25–27-gauge needle can be used but aspiration of blood must be done slowly to avoid collapsing the vessel. Serial venipuncture is possible.

The submental collection technique is a reliable and quick method for serial blood sampling in mice. The technique involves puncture of the submandibular vascular bundle (inferior labial vein and facial vein) under the chin in mice, halfway between the ventral hair whorl (blue circle in below image) and corner of the mouth, using a disposable, sterile single-use mouse bleeding lancet. The blood vessels (red solid dots in below image) may be visible under the skin when the area is shaved. The inferior labial vein runs along the dorsal border of the mandible, parallel to the ventral edge of the buccinator muscle. The facial vein runs along the masseter muscle on the ventral side and crosses the mandible along the rostral border of the masseter. The maximal blood volume obtainable is approximately 10% of a mouse's circulating blood volume, and hemostasis can be achieved with gentle compression at the punctured site.





Tail tip amputation is performed under anesthesia, when a small piece (1-2 mm) of the distal tip of the tail is removed. **This method is discouraged.** Repeated blood samples can be obtained by removing the scab. **The maximum length of tail that can be removed is 3 mm.**

Cardiac puncture technique is limited to an acute, terminal collection due to the technical difficulty and the danger of lung injury or cardiac tamponade and death. Safer alternative techniques are available for serial sampling. Cardiac puncture should be done under general anesthesia or post-euthanasia with CO2 in rodents. Using a 1 ml syringe or larger, and a 23–26-gauge needle, insert the needle just under the xiphoid process (caudal tip of the sternum), directing the needle straight along the midline and at 30-45° angle to the animal. Aspirate gently until blood is seen in the syringe. In adult mice, it is possible to get about 1 ml of blood, in rats 5 mls or more.



Table of Blood Collection Sites in Mice and Rats

Note: All frequency recommendations assume a skilled operator and no complications.

Route	Anesthesia required	Amount collected	Repeated sampling	Tissue damage	Recommended M (mouse), R (rat)	Materials needed	Comments
Cardiac puncture	Yes	+++ 1ml (M), 5-10 ml (R)	No	Moderate	For terminal collection of large volume of blood M/R	1-3 cc syringe, 23-25 g needle	Once
Dorsal pedal vein	No	+	Yes	Low	M/R	25-27 g needle, microhematocrit tube	Can repeat daily (remove scab), alternate legs
Jugular vein	Yes	+/++	Yes	Low	M/R	0.5-3 cc syringe, 25-27 g needle	1-2 times/week, alternating sides
Lateral tail vein	No	+/++ 0.1-0.15 ml (M), up to 2.0 ml (R)	Yes	Low	M/R	25-27 g needle, 0.5- 3 cc syringe, microhematocrit tube	Can repeat daily (remove scab), alternate sides and sites along tail
Retroorbital sinus	Yes	+++	Yes	Moderate /high	M (with caution)	Microhematocrit tube	Rotate eyes and can repeat only if eye looks normal
Saphenous vein	No	++ 0.1ml (M)	Yes	Low	M/R	23-27 g needle, 1-3cc syringe, microhematocrit tube	Can repeat daily, alternate legs and sites along vessel
Submandibular vascular bundle	No	++ 0.2-0.5 ml	Yes	Low	M	Lancet 4-5.5 mm	Alternate sides and can repeat only if venipuncture site look normal
Submental	No	++ 0.2-0.5 ml	Yes	Low	M	Lancet 3-5mm	Alternate sides and can repeat only if venipuncture site looks normal
Tail tip amputation	Yes	++ 0.1 ml	Limited	Moderate	M	Scalpel blade	No more than 3 mm

Examples of Blood Volumes That Can Be Withdrawn

Body weight	Total Blood Volume (ml); 6-7% of body weight	Maximum draw allowed in a 2-week period (15% of Total Blood Volume)
15 g	0.9 - 1.1	< 0.16 ml
20 g	1.2 - 1.4	< 0.21 ml
25 g	1.5 - 1.8	< 0.26 ml
30 g	1.8 - 2.1	< 0.31 ml
150 g	9.0 - 11	< 1.6 ml
200 g	12 - 14	< 2.1 ml
250 g	15 - 18	< 2.6 ml
300 g	18 - 21	< 3.2 ml

References:

Hayward AM, Lemke LB, Bridgeford EC, Theve EJ, Jackson CN, Cunlijfe-Beamer, Marini RP. 2007. Biomethodology and surgical techniques (Chapter 13) . In: Fox JG et al editors. The mouse in biomedical research, Volume III, 2nd edition. Academic Press.

Diehl KH, Hull R, Morton D, Pfister R, Rambemampianina Y, Smith D, Vidal JM, Vorstenbosch C. 2001. A good practice guide to the administration of substances and removal of blood including routes and volumes. J. Appl. Toxicol. 21: 15-23.

Hoff J. 2000. Methods of Blood Collection in the Mouse. Lab Animal 29(10): 47-53.

Popesko P, Rajtova V, Horak J. 1992. A color atlas of the anatomy of small laboratory animals. Boca Raton (FL): CRC press.

Regan RD, Fenyk-Melody JE, Tran SM, Chen G, Stocking KL. Comparison of Submental Blood Collection with the Retroorbital and Submandibular Methods in Mice (Mus musculus). J Am Assoc Lab Anim Sci. 2016;55(5):570-6. PMID: 27657712; PMCID: PMC5029828.

Cage Card System

Rodent and other small animal cages utilize a cage card system to convey information relating to the cage. Printed cage cards can be inserted into the holders on the cages. **Every cage must have a cage card indicating the Principal Investigator (PI), current CAC protocol number, account number, and contact information (name, email address, and cell phone number).**

In addition, other cage cards and/or a sticker system are used for communication between DCM staff and researchers. Details on each card can be found in the DCM Cage Cards ecourse.

Current Contact Information Required

It is REQUIRED that accurate and current contact information for lab members is readily available for EVERY animal. This information is most often reported on a cage card, a "Contact Information" sheet posted on a cubicle door, or, for larger animals, in the medical record; untidy notes are not acceptable. **Contact information includes full name, email**

address, and cell phone number. Lab personnel should notify DCM staff when they go on holiday and who will be responsible for animals.

When animal users leave MIT or WIBR, they must inform the CAC (cacpo@mit.edu) and DCM (dcmaccess@mit.edu).

Experimental Endpoints

To assure that humane animal care is provided, experimental endpoints must be clearly defined in the CAC protocol. See the CAC policy "Experimental Endpoints" for more information, including a list of generally acceptable appropriate endpoints and additional endpoints for tumor studies.

Death as an endpoint is unacceptable unless strongly justified scientifically.

Euthanasia Methods

The use of euthanasia techniques suitable to each species is mandated by the federal government through the AWA and the Health Research Extension Act of 1985. The 2020 "AVMA Guidelines on Euthanasia of Animals" is used for evaluating euthanasia methods and in directing the choice of euthanasia by investigators.

- **Normal cull rodents should be euthanized by DCM using the Euthanex system. "To Euthanize by DCM"** cards can be filled out and placed on cages of clinically normal cull rodents. These animals should be deducted from your cage census on the holding room clipboard.
- **Do not place a "To Euthanize by DCM" card on any cages that have active Health Check cards on them.**
- Do not remove pups and place them alone in a cage OR remove the dam from a litter unless pups are >21 days (i.e., weaning age).
- Rodents are euthanized by DCM approximately weekly. Breeding cages with weaning age pups should be separated prior to marking the cage for DCM to euthanize to avoid overcrowding.

All personnel euthanizing individual cages of mice and rats with CO_2 (including sick animals and animals euthanized by laboratory personnel) must follow the instructions posted at CO_2 stations within the animal facilities and lab spaces approved for euthanasia.

Field Investigations

CAC protocol approval is required in advance for some studies in outdoor environments that involve non-domesticated species (i.e., wildlife). See the "Field Investigations" policy for additional information.

Genotyping of Mice

Genotypes of genetically engineered mice (GEM) colonies should be checked routinely to confirm genetic identity and avoid costly experimental errors. DCM training in genotyping methods can be obtained by contacting dcmtraining@mit.edu. All genotyping procedures must be described in a CAC-approved protocol. See "Genotyping Rodents" for more information.

Using Transnetyx for Genotyping

DCM contracts with Transnetyx to provide offsite genotyping of knock-out and knock-in mouse strains. Transnetyx provides labs with accurate, timely, and efficient genotyping. DCM encourages the use of Transnetyx for any lab, but it can be particularly beneficial for labs that need to keep up with genotyping a large number of animals. Ear punches or tail tips are loaded into specially designed well plates obtained from one of four "TAG Centers" located in building 16/56, 8th floor; the basement of building 68 between 68N and 68S; the 7th floor of building 76 outside the elevator; and the 7th floor of building 46, just outside the "red" section elevator. Strain and ID number are recorded on a corresponding information card at the time of biopsy. The full well plate is dropped back into one of the TAG centers for shipment. The researcher keeps the information card and uses it to submit an order for genotyping online.

Well plates are picked up from all TAG Centers after 2 pm on Mondays and Thursdays; they are shipped for next day delivery to the Transnetyx headquarters where they will be analyzed.

Results are available online to the researchers within 24 or 72 hours after arrival at Transnetyx (pending selected service level).

Handling and Restraint of Animals

Proper handling of animals is imperative to good science, good data and good animal welfare. The proper handling of animals is trained during the orientation to the species, and training can in methods of proper handling and restraint can be provided by DCM for all species utilized on campus. Please contact dcmtraining@mit.edu for specific training requests.

Restraint refers to manual restraint and the use of restraint devices. Routine restraint for the purpose of drawing blood, giving an injection or examining an animal causes minimal distress to the animal if properly performed. Non-routine restraint (restraint of an animal for longer than 15 minutes, e.g., chair restraint in behavioral research) requires special consideration and approval by the CAC. All procedures employing restraining devices will be limited to a maximum of 10 consecutive hours. The use of restraint for periods less than 10 hours must be justified in the animal use protocol and approved by the CAC during protocol review. The CAC will prescribe stipulations for restraint use which may include release from restraint for specified periods. See the CAC policy "Prolonged Restraint."

Note that anytime animals are handled, the handler must wear disposable gloves along with other appropriate PPE. Animals in restraint devices should never be left unattended and the restraint device must be completely sanitized after use.

Injections and Inoculations

The CAC/EHS require drugs, needles and syringes to be kept secured at all times, either in a locked laboratory cabinet or in locked laboratory space and not left unsecured in animal rooms, procedure rooms, or laboratories.

Needles should not be reused for multiple injections as reuse leads to dulling of the needle, increasing the discomfort associated with injections for the animal, and can lead to disease transmission and/or contamination of vials of material to be injected.

Needles should never be recapped and must be discarded only in sharps containers.

Injections

The route chosen for an injection will depend on the species, volume and material to be injected, and the desired rate of absorption. The investigator should know the physiological properties of the substance to be injected because tissue damage and discomfort can be caused by irritating vehicles or drugs. Accurate placement of the needle should always be determined prior to the actual injection by applying slight negative pressure on the syringe and observing for flashback of blood or other body fluid. Repeated injections by the same route should be done using alternative injection sites (e.g., using alternate eyes for serial retro-orbital injections) and should not be done if there is tissue damage from prior injections.

Common, Acceptable Routes of Administration of Substances:

INTRAPERITONEAL (IP) INJECTION

This is a commonly used method for injection in small rodents because they have limited muscle mass and relatively small veins. The animal should be held in dorsal recumbency with the head pointing downwards. Intraperitoneal injections are given in the lower left or lower right quadrant of the abdomen as vital organs are absent in these areas. To prevent injection into the intestine, only the tip of the needle should penetrate the abdominal wall. Slight negative pressure should be applied to the syringe prior to injection. If any bodily fluid is pulled into the needle hub (such as urine), the syringe should be discarded, a new syringe prepared, and the procedure repeated. Injections should be made slowly to avoid bruising of tissue and hemorrhage from the pressure of the spray. Excessive volumes should not be administered by the intraperitoneal route (not >1-2 ml in rodents). Intraperitoneal injections are discouraged in the rabbit.

INTRAVENOUS (IV) INJECTION

The lateral tail vein is frequently used for intravenous injection in small rodents. Skin should be wiped with alcohol prior to injection. A 26-gauge needle or smaller is used for venipuncture in mice and a 23-gauge needle or smaller in rats. Injection of tail veins is generally accomplished with compression of the lateral tail vein by applying pressure at the base of the tail. Warming of the tail is useful to increase blood flow. In rabbits, ear veins, tarsal veins and cephalic veins are common sites for intravenous injection. Ear, cephalic and saphenous veins are also useful sites in guinea pigs. Prolonged intravenous administration or sampling can be performed by

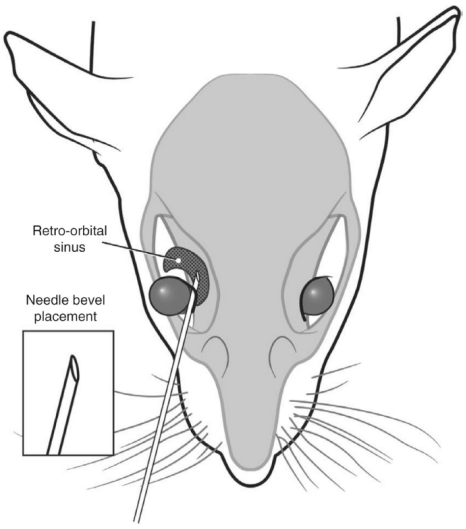
catheterization of the blood vessel. Procedures to ensure hemostasis after injection need to be employed.

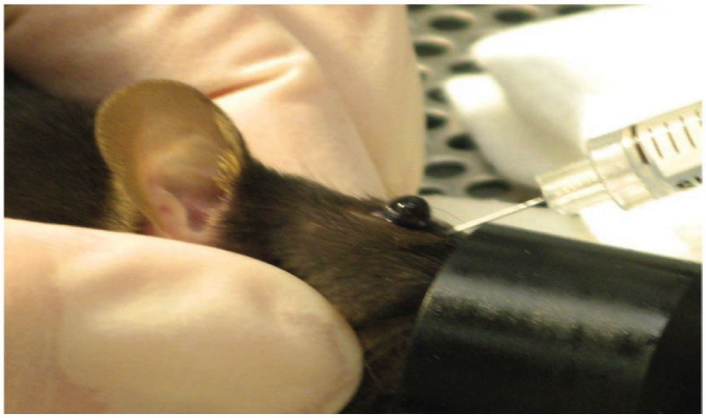
SUBCUTANEOUS (SQ) INJECTION

In most laboratory animals the most convenient site for a subcutaneous injection is the back of the neck between the scapulae. A fold of skin behind the neck is held between the fingers and a needle is inserted under the skin at the base of the fold.

RETROORBITAL INJECTION

This is also a common method for intravenous injection in mice. Animals should be properly restrained and general anesthesia is required for injections into the retro-orbital sinus. In addition, a drop of ophthalmic topical anesthetic can be placed on the eye that will receive injection to provide additional analgesia. 27 gauge or smaller needle is preferred for the retroorbital injection. The needle is carefully introduced with bevel facing down and at an angle of 30-45° into the medial canthus. Direct the needle along the edge of the eyeball down until the needle tip is at the base of the eye. Slowly and smoothly inject the substance and then withdraw





the needle gently. There should be no or little bleeding. **Tumor cell lines should never be injected in this route as accidental spilling of cells can lead to tumor development in the retroorbital space.**

(Figures adapted from Yardeni et al., 2011. Retro-orbital injections in mice. Lab Animal (NY) 40(5): 155-160.

INTRAMUSCULAR (IM) INJECTION

The most commonly used sites for intramuscular injections are the muscles of the thigh, and the quadriceps muscles in front of the femur are most safe.
Intramuscular injections are not used routinely in mice due to their small muscle mass. In larger species such as guinea pigs and rabbits, the lumbar muscles lateral to the vertebrae can also be used for intramuscular injections. Excessive volumes (>1 ml in the rabbit) should not be given in a single intramuscular site (see table below).

INTRADERMAL INJECTION

Intradermal injections are typically used to assess immune or inflammatory responses. The injection site should be shaved to improve visualization. Very small volumes (typically 0.05ml) can be administered by inserting a needle parallel to the skin, bevel up just under the superficial layer of the epidermis. The needle is advanced three to four millimeters, and the substance is injected. Some resistance should be felt, and a bleb should become visible in the skin. When the needle is removed, slight pressure is applied to the injection site to prevent leakage. Footpad injections are discouraged and must be scientifically justified. A common alternative site in rodents is the caudo-medial aspect of the hock. In non-rodent species, larger injection volumes may be administered at multiple sites. Please consult veterinary staff regarding location of intradermal injections in other species.

Intradermal (ml/site)	
Mouse	0.05
Rat	0.05
Rabbit	0.05
Macaque	0.1
Minipig	0.1
Marmoset	0.05

INTRACRANIAL INJECTION

Intracranial injections are utilized to facilitate compounds, substances or cells into the brains of animals. This may be done in adult animals as a surgical procedure (craniotomy) or in neonatal animals through the intact skull. Regardless of the age, intracranial injections must be done at a slow and deliberate flowrate to minimize the amount of reflux from the sight and to allow intracranial pressure to be maintained. A flow rate of 0.05-0.6 µl/minute is recommended using a Hamilton non-coring needle on an automated micropump. The researcher should wait 5 to 10 minutes after injection before removing the needle to minimize reflux. The maximum volume for intracerebral administration in mice is <0.002 ml (<2 µl) per deposit (Keiser, M. S., Chen, Y. H., & Davidson, B. L. (2018). Techniques for intracranial stereotaxic injections of adeno-associated viral vectors in adult mice. Current Protocols in Mouse Biology, 8, e57) In addition, for adult rodents, it has been suggested that the volume "should not to exceed 2%" of the brain volume (Joint Working Group on Refinement, *Refining Procedures for the Administration of Substances*, *Laboratory Animals* 35: 1-41, 2001).

INTRATRACHEAL / INTRANASAL INSTILLATION

For intratracheal instillation of substances, general anesthesia is recommended. Animals should have their oropharynx opened to facilitate view of the epiglottis and associated trachea. In general, this should be performed in a biosafety hood to decrease risk to personnel, based on EHS recommendations. For intratracheal instillation, a volume of 50 to 125 µl can be used per mouse; however, a maximum of 75 µl (total volume per mouse) is recommended. A total volume of 125 µl should not be used in young mice (less than six weeks old). If a total of 125 µl needs to be used, the volume should be divided into two doses of 62.5 µl each; wait for one to five

minutes after the first dose before giving the second. After giving the first dose, the respiratory rate and pattern of the mouse should return to normal before giving the second dose.

For intranasal installation, anesthesia is not required. Dosing within a biosafety hood also provides personnel protection, based on EHS recommendations. In general, no more than 25-50µl per nostril is suitable for intranasal instillation and is species dependent.

OROGASTRIC GAVAGE

Use ball-tip stainless steel or disposable plastic feeding needles are utilized to instill compounds into the stomachs of animals, namely rodents. These specialized feeding needles are designed to decrease the potential of iatrogenic oropharyngeal tissue damage and prevent the inoculation of substances into the trachea. The feeding needle / gavage tip MUST be measured from the opening of the mouth to the area of the sternum/rib cage to ensure that stomach perforation does not occur. The animal (mouse, rat) should be firmly grasped and not allowed to move from side-to side, as this increases the chances of misplacement of the feeding needle. The feeding needle should be passed into the commissure of the mouth, in the diastema between the teeth. The feeding needle should smoothly pass into the esophagus. If any resistance is felt, the needle should be immediately removed as this runs a risk for tracheal injury, esophageal injury and/or subcutaneous emphysema. Once the substance is expelled into the stomach, the feeding needle should be removed, and the animal closely monitored for any signs of compromise, labored breathing, or other signs of injury. For mice, a 27-24g 1-1.5" straight or bent feeding needle is recommended and ≤ 0.2 mL of substance can be administered intragastrically. For rats, an 18-22g 3" straight or bent feeding needle is recommended and ≤0.5 mL of substance can be administered intragastrically.

INJECTIONS IN FROGS

Injections into the dorsal lymph sac are common in frogs (see picture below). Based on the size of the frog, a 22 to 28 gauge needle, ½ to ¾-inch length should be used to inject 0.5 ml or less. With the needle placed almost parallel to the frog's body and just to the middle of the frog's lateral line, penetrate the skin with the needle bevel facing up. The large musculature of the rear legs in front of and behind the femur can be used for intramuscular injections in frogs. Using a 22 gauge needle with ¾-inch length, a volume of 0.15 to 0.5 ml (depending on the size of the frog) can be administered easily. Intracoelomic injections in frogs are similar to intraperitoneal injections in mammals.

For injections in other species, including zebrafish, consult the DCM veterinary staff.

Injection Volumes

Dosing Volumes Considered Good Practices (and Maximal Dose Volumes)[a]

Species	Route and Volumes (ml/kg)					
	Oral	SQ	IP	IM	IV (bolus)	IV (slow injection)
Mouse	10 (50)	10 (40)	20 (80)	0.05[b] (0.1)[b]	5	(25)
Rat	10 (40)	5 (10)	10 (20)	0.1[b] (0.2)[b]	5	(20)
Rabbit	10 (15)	1 (2)	5 (20)	0.25 (0.5)	2	(10)
Macaque	5 (15)	2 (5)	[c] (10)	0.25 (0.5)	2	[c]
Minipig	10 (15)	1 (2)	1 (20)	0.25 (0.5)	2.5	(5)
Marmoset	10 (15)	2 (5)	c (20)	0.25 (0.5)	2.5	(10)

[a]For non-aqueous injectates, consideration must be given to time for absorption before re-dosing. No more than 2 intramuscular sites should be used per day, and subcutaneous sites should be limited to 2 or 3 sites per day.

[b]Values in mls per site (not ml/kg).

[c]Data not available.

Table adapted from:
Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, and van de Vorstenbosch C (2001): A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol, 21(1):15-23.

Needle Gauge Recommendations

	SQ injection	IM injection	IP injection	IV injection
Mouse	25 gauge	27 gauge	25-27 gauge	26-31 gauge
Rat	25	25-27	23-25	23-25
Hamster	25	25-27	23-25	25-27
Guinea pig	23-25	25-27	23-25	25-27
Rabbit	21-25	25	21-23	23-25
Ferret	21-23	23-25	21-23	21-25
Sheep	18-20	21-23	Not done	18-22
Swine	18-20	21-23	Not done	18-22
Macaque	21-25	23-25	Not done	21-25
Marmoset	23-25	25-26	Not done	25-26

adapted from *Formulary for Laboratory Animals*, 3rd Ed. Hawk, C et al

Injection Routes That Are Discouraged

The following routes of injection are discouraged and require scientific justification from the investigator and approval by the CAC:

- Intraarterial injection - into an arterial blood vessel
- Intracardiac injection - into the heart
- Footpad injection - into the pad of the foot

Contact DCM veterinary staff for more information about these techniques.

Injecting/Implanting Cell Lines: Pathogen Screen Required

When cells or cell lines (human or rodent), especially from outside sources, are administered to mice or rats as part of a CAC-approved protocol, there is a concern that the cell lines may be infected with murine pathogens, e.g., mouse parvovirus, mouse hepatitis virus or lymphocytic choriomeningitis virus.

To protect the health status of rodent colonies at MIT/WIBR, all cell lines that are brought in from any outside source **(including commercial sources such as the ATCC, which do not routinely test for murine pathogens),** or that originated from animals in a different MIT/WIBR colony room than the recipients, require initial pathogen testing before use. Rodent or human cell lines that have been re-passaged in rodents for more than five years at MIT/WIBR must also be tested.

The researcher is required to submit such cells to DCM for PCR testing before they can be used experimentally; contact the Manager of Diagnostic Services dcm-diagnosticlab@mit.edu to make arrangements. Up to five different cell lines can be pooled into one sample for testing, with the understanding that any positive results will require further testing. Pending test results, projects may start under quarantine housing only when pre-arranged in advance with the Animal Resource Manager. An Attachment D must be filed with the CAC protocol for approval to administer cells.

Fresh human cells or rodent cells or tissues harvested from within the same MIT/WIBR colony room and being used as a single passage in rodents do not require pathogen testing. (This policy assumes that the health status of your rodent colony has not been compromised by a pathogen break. If in doubt, consult the veterinarian for your colony.)

Polyclonal and Monoclonal Antibody Production

Polyclonal or monoclonal antisera that are commercially available as a catalogue item **and will not be used in animals** at MIT/WIBR are not a concern of the CAC or DCM and do not require a CAC protocol. However, if the investigator requests another institution (commercial or academic) to produce unique antisera, advance CAC approval is required. See the "Antibody Production" policy on the CAC website for more information.

Species-Specific (Special) Guidelines

Avian Eggs

The CAC requests notification about research use of avian eggs (typically chicken, *Gallus domesticus*, due to the potential for hatching. Eggs must be purchased SPF (Specific Pathogen Free) for *Salmonella s*pp, used prior to approximately 15 days from laying and a plan for euthanasia of hatched eggs with notification to the CAC must be in place.

Fish Embryo and Larvae Use

Finfish are vertebrates, and thus fall under the scope of the PHS Policy, The Guide and other associated regulations, such as the US Government Principles. Most typically, the term "fish" refers to zebrafish or other small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic fish at MIT. The PHS Policy endorses that the life stage at which these fish hatch out of the chorion, is when fish should be "counted" as a vertebrate animal.

In general, zebrafish hatch at 3 days post fertilization (dpf). The age at which hatching out of the chorion may vary from species-to-species, and therefore should be clarified in the CAC protocol application form for other species of fish, if used.

See the "Fish Embryo and Larvae Use" policy on the CAC website for guidelines on fish use.

Amphibian Embryo and Larvae Use

Amphibians (frogs, axolotls etc.) are vertebrates and thus fall under the scope of PHS Policy, The Guide and other associated regulations, such as the US Government Principles. The CAC's definition of "amphibian" includes embryos and larvae of any age, so any procedures performed on these young animals must be described in an approved animal use protocol before implementation. Unfertilized eggs do not require a full animal protocol, but a Tissue Harvest Exemption should be in place for their use at MIT. See the "Amphibian Embryo and Larvae Use" policy on the CAC website for guidelines

Satellite Facilities

Laboratories planning to use aquatic animals (e.g., frogs or zebrafish) or birds outside of a DCM facility, must first meet with the DCM Director or their designee to assess the suitability of the proposed Satellite Facility space. Once cursory approval is granted, the CAC must also provide approval for the suitability of the space. PIs are expected to complete and submit an SOPs for husbandry practices and provide an emergency plan to the CAC (as per the *Guide for the Care and Use of Laboratory Animals,* 2011) with a copy of the protocol. Once approved, the emergency plan should be laminated and posted in the housing area. Areas will be inspected by both the CAC and visited by DCM veterinarians to ensure the care and welfare of animals; the Satellite Facility is maintained as expected, and there are no deviations from standard practices. Templates for these SOPs are available from DCM; contact DCM training for copies.

Behavioral Management of Nonhuman Primates

DCM follows the requirements of the USDA Animal Welfare Regulations (AWRs) and has established a Program for the Behavioral Management of Nonhuman Primates, which is filed annually with the CAC. Consistent with the AWRs, these important topics are addressed:

- Social grouping: Default pair or group-housing of nonhuman primates.
- Environmental enrichment: Cage enrichment, videos, diet, positive interaction with human personnel, feeding enrichment, learning tasks in the research lab.
- Special considerations: Behavioral assessments performed at regular intervals, or more often if needed.
- Restraint devices: Cannot be used for more than 10 consecutive hours.
- Exemptions: Require justification as to why an animal should be individually housed and/or not receive enrichment.

Contact the DCM behavior staff or the CAC for more information.

Transporting Animals

When an animal is to be moved within its holding room, or elsewhere, it must be safely restrained, e.g., a microisolator top must be in place for mouse cages. Care must also be taken that the water bottle is turned upside down for long transports to prevent leakage (and flipped back again when returned!).

When transporting animals to a research lab, the animal cage(s) must be covered completely with an opaque material. An animal can only be transferred from one holding room or facility to another with the approval of DCM, as described below. **Live animals may be removed from animal facilities only if doing so is described in an approved CAC protocol.**

Between or Within DCM Animal Facilities

For the transportation of rodents to another holding room or animal facility within MIT/WIBR, fill out a request online.

To Research Labs

Transport of animals to research labs is STRONGLY DISCOURAGED for procedures that can be performed within the animal facility, e.g., necropsies and surgeries. Performing procedures within the facility minimizes animal stress and potential cross-contamination. In addition, feral rodents are attracted to research animals, caging, food, and water.

Regular transport to research labs, e.g., for behavioral testing, must be described in an approved CAC protocol. As noted above, animals must be safely restrained and completely covered during transit. CAC expectations for laboratory spaces to which animals are brought are described in an earlier chapter.

Note that any vehicle used to transport animals must be climate-controlled and approved by the CAC; public modes of transportation (such as subway or taxi) can never be used for the transport of research animals.

Tumor Guidelines in Mice and Rats

The CAC has standard euthanasia criteria for tumors in rodents (see the CAC policy "Experimental Endpoints"). Standard endpoints are BCS < 2, tumor ulceration, aggregate tumor size of 1 cm in mice or 2 cm in rats, or impairment in eating, urination, defecation, or ambulation. Exceptions to these criteria, particularly size or ulceration, may be needed for a particular study or cell line. Exceptions should ideally be discussed with the facility veterinarian and must be scientifically justified and approved in the CAC protocol. When exceptions are requested, alternative endpoints must be provided.

Chapter 6: DCM Animal Facilities

List of Animal Facilities and Traffic Patterns

DCM animal facilities vary in animal health status. The potential for cross contamination between animal colonies by traveling from one building to another is a real concern. As a result, specific traffic patterns between buildings must be followed. **Facilities are listed below in the order in which they may be entered ("cleanest" to "dirtiest"):**

Facility Name, Species (cleanest --> dirtiest)	**Address**
CLEANEST: 68N, subbasement (mice)	31 Ames Street
46TG, 7th floor (mice)	43 Vassar Street
WIBR, 2nd floor (mice, fish, Axolotls)	455 Main Street
76, 7th floor (rodents)	500 Main Street
68S, subbasement (mice)	31 Ames Street
E17/18, 6th floor (multiple species)	40 Ames Street
E25, basement (multiple species)	45 Carlton Street
46NHP, 7th floor (multiple species) / 56, 8th floor	43 Vassar Street / 21 Ames Street
DIRTIEST: Quarantine	

Within each facility, there is a room entry order that must be followed. Refer to the room entry order signs posted in each facility.

No researcher can enter a DCM animal facility without CAC and DCM approval.

DCM will not allow anyone who has entered another institute's animal facility to enter a DCM animal facility for 48 hours after they have been in the outside facility.

Types of Spaces in Animal Facilities

Rodent Barrier

68N is a dedicated barrier facility that houses only embryo transfer-rederived animals or animals from an approved vendor. Animals housed in this facility are of an improved and more highly defined health status, in addition to being specific-pathogen free (SPF).

Specific-Pathogen Free Rooms

All animals maintained in MIT/WIBR facilities are specific pathogen-free (SPF), except for the few animals of unknown health status that are maintained under quarantine conditions. Each SPF room or cubicle contains animals free of defined pathogens that may vary between locations. Procedures used to exclude unwanted pathogens include facility and room entry order, traffic separation by two corridors (clean and dirty), filter-top cages, vented cage racks, mass air-displacement rooms (cubicles), and PPE requirements.

Behavior Spaces

DCM behavior spaces are located within the 46Tg, 46SA, and E17/18 facilities.

Containment Areas

Containment Areas (CAs) have individual cubicles for animals that receive hazardous materials such as toxic chemicals (e.g., carcinogens), infectious agents, or radioisotopes. Access is restricted and appropriate signs are posted. Wearing of disposable coats, shoe covers, gloves, hair covers, and masks (plus respirators when indicated by MIT/WIBR'S EHS) is required. Special procedures are required for egress of supplies and personnel (when indicated). Live animals are removed from CAs only with approval from the DCM Hazards Coordinator and/or the Animal Resource Manager.

Researchers must be cognizant of the risks involved in working with hazardous materials in animals. It is their responsibility to ensure safety of all personnel during the project and follow the guidelines and procedures provided by DCM.

Imaging Area

An Imaging Area (76IA) is located in 76; MRI, ultrasound, microCT, PET, and IVIS imaging equipment are provided in the 76IA. Lab members must be trained on the use of all equipment by the Preclinical Imaging and Testing Facility team (AIPTcore@mit.edu). Imaging of animals must be approved on a CAC protocol and a separate imaging suite orientation will be given before individuals have access.

Procedure Rooms

These rooms are designed and equipped for animal manipulations. Each room has either slotted vent hoods or vented tables. Most of these rooms are equipped with a CO_2 chamber and a CO_2 source for euthanizing small animals. Anesthesia machines are available in some procedure rooms in each facility.

Procedure spaces and animal holding rooms have different types of air flow units that provide protection. The correct type of "hood" must be used based on the materials and/or animals you are working with.

- Animal Transfer Stations (ATS) are located in the animal rooms and are required for to be utilized when handling mice, rats or other small mammals.

- Laminar flow hoods offer "product" protection only. HEPA-filtered ("high efficiency particulate air") air flows from the rear of the hood across the work area. The unidirectional flow of air prevents cross contaminating animal cages that are side-by-side on the work surface. **Volatile liquids like isoflurane cannot be used in laminar flow hoods.**

- Biological safety cabinets (BSCs) are for use with hazardous biological and chemical agents. **Volatile liquids like isoflurane can be used in ducted BSCs (ducted to the outside) or chemical fume hoods.**
- Chemical fume hoods are appropriate for the use of volatile liquids and certain hazardous agents.
- Counter surface slotted vent hoods are for volatile anesthetics, i.e., isoflurane, only and not for any other hazardous agents.

Necropsy Rooms

Each animal facility has a designated space for the postmortem dissection of animals. This may be facilitated via procedure room spaces, or dedicated necropsy areas. These necropsy rooms are equipped with a CO_2 chamber and a CO_2 source for euthanizing small animals, refrigeration facilities for storage of dead animals, and specially designed dissection tables.

Scheduling use of these rooms may be necessary to avoid conflicts; in that event a sign-up sheet will be posted on the necropsy room door by DCM. Waste receptacles are provided in the room for dry waste, and plastic bags are provided for carcasses, body parts, and fluids. This bagged material should be placed in the refrigeration chamber provided for that room. All waste will be collected by DCM personnel and sent out for incineration. All hazardous waste must be placed in biohazard bags and stored for appropriate disposal.

Quarantine

The term "quarantine" refers to rooms, cubicles, cabinets, or isolators used to hold animals for observation or determination of health status. Such facilities are used primarily to condition and monitor newly received animals and to isolate animals either suspected of harboring undesirable infectious agents or of unknown health status. **Access is restricted**.

For anyone using both specific-pathogen free (SPF) and non-SPF (quarantine) animals in the same day, the quarantine animals must be worked with after completing work with the SPF animals. The investigator must shower and have a clean change of clothes before working with SPF animals the following day.

To maintain SPF status, any animals removed from MIT/WIBR animal facilities should not be returned to those facilities (except for pre-approved studies). Special arrangements for transporting animals to and back from laboratories or outside institutions must be arranged through the DCM veterinary staff.

Support Spaces and Equipment

Each animal facility is equipped with areas and equipment to provide support for various animal manipulations and/or special handling requirements. Continued availability of support areas and special equipment requires that investigators leave them clean after each use. **Syringes, needles, and drugs must be kept stored in locked cabinets and not left unsecured in animal rooms,**

procedure rooms, or laboratories; any needles or syringes left in support areas will be confiscated. Any investigator supplies stored in support areas must be kept neat, organized and labeled with contact information.

- Special diet storage. Cold rooms/refrigerators are available in each facility for storage of investigator's special animal diets or ingredients. These diets must be clearly labeled with the investigator's contact information and expiration date of the diet. Nothing may be stored in the animal facility without permission of DCM.

- Euthanasia chambers. CO_2 euthanizing chambers and detailed instructions for performing euthanasia are provided in all necropsy rooms and procedure rooms.

- Euthanex systems. Trained DCM personnel operate Euthanex machines in 68N, 46TG, WIBR, 76, 68S, and 46SA. These machines anesthetize animals before they are euthanized and thus provide added assurance of a humane death. Magenta-colored "To Euthanize by DCM" cards are placed on cages by investigators who request this service. **Animals with health check cards cannot be euthanized in a Euthanex machine, i.e., the animals must be clinically normal and healthy.**

Housing Assignments for Animals

All animals at MIT/WIBR must be housed in DCM animal facilities or in rooms approved by the DCM's Director and the CAC. Animal housing is determined by the species, the type of microenvironment required, the type of equipment which may be needed, and the microbial status of the animals. DCM's management of the micro- and macroenvironment helps to protect animals from exposure to infectious diseases or environmental factors that can interfere with research. Animal holding room assignments are also made on the basis of species, research objectives, number of animals, microbial status of the animals and proximity to investigators' laboratories. Normally, animals are housed one species per room/cubicle and, when possible, from one vendor source.

DCM's Animal Resource Managers assigns space with appropriate equipment. The type of caging equipment and classification of holding space (short term, long term, quarantine, containment, etc.) is dependent on the nature of the study.

For security reasons all facilities are restricted to authorized personnel only by access cards. To arrange for access to the facility and rooms that your animals are housed in, a request must be made to dcmaccess@mit.edu. Investigators must be CAC certified before receiving access.

Any requests to house animals outside DCM's designated facilities (>12 hours) must be first discussed with DCM's Director, and approval must be granted in writing by the CAC. Satellite facilities (e.g., fish or finches, housed in investigator labs) must be described in an standard operating procedure (SOP) husbandry template approved by DCM and the CAC.

Housing Space Requirements and Overcrowded Cages

It is an institutional responsibility that clean, dry, comfortable housing be provided to animals used in research. This is essential for meeting regulatory guidelines, to provide healthy animals for research, and, importantly, to promote the animals' welfare.

Current space recommendations for laboratory animals are based on the best available information as defined by the current edition of the *Guide for the Care and Use of Laboratory Animals (Guide).* DCM recommendations below conform to these requirements. The following tables, largely excerpted from the *Guide*, provide space requirements for select animal species.

Maximum Number of Mice Allowed per Cage

Mouse weight (g)	Small Cage (11.4 x 6.6 x 5 in)	Large Cage (17 x 9 x 6.5 ⅛ in
<10	10	22
10-15	8	16
15-25	**5**	**11**
>25	5	9
Female and litter	1 female + litter	Up to 2 females + 2 litters + 1 other adult mouse

Maximum Number of Rats Allowed per Cage

Rat Weight (g)	Cage size (17 x 8.5 x 8 in)
<100	8
100-200	6
200-300	4
300-400	3
400-500	2
>500	1 (move to larger enclosure preferred)
Female with Litter	1 female + litter (move to larger enclosure preferred)

Mouse and Rat Breeding Practices and Animal Density Expectations

At MIT/WIBR, mice are the predominant species used in research and are frequently bred in-house. Investigators are responsible for monitoring their animal populations to prevent overcrowding and ensure appropriate animal housing conditions.

Investigators must assess animal numbers and cage density at least once every seven days. This assessment should include reviewing the animals in each cage and checking the Animal Health Monitoring sheet posted on the room door.

Breeding Practices

Based on DCM experience and the animal density recommendations outlined in the CAC Policy on Rodent Breeding, monogamous pair breeding is strongly recommended. Breeding cages should generally contain no more than one female with a litter per cage. The female may remain housed with a male.

Harem breeding (2 females/1 male) arrangements may be used for mating purposes; however, females must be separated before pregnancy is carried to term. This practice helps prevent overcrowding associated with multiple pregnant females and litters within a single cage.

Weaning

Mouse and rat litters should generally be weaned from their mothers at 21 days of age. Certain less hardy or mutant strains may require delayed weaning.

Delayed weaning may be permitted under the following circumstances:

- The need for delayed weaning is specifically justified and approved by the CAC as part of the animal use protocol.
- The DCM veterinary team may approve delayed weaning on a case-by-case basis when warranted by the strain or other animal-related considerations.

Animal Density and Identification of Overcrowded Cages

Cages will be considered potentially or definitively overcrowded based on the following criteria:

Breeding cages:

- Cages containing more than one pregnant female will be considered potentially overcrowded.
- Cages containing more than one litter will be considered overcrowded.
- Cages containing a litter that has reached weaning age will be considered overcrowded if the litter has not been appropriately weaned.
- Cages containing timed-pregnant females that are not intended to carry their pregnancies to term must be clearly labeled to indicate this status and avoid being incorrectly identified as overcrowded.

Non-breeding cages:

- Small cages containing more than five mice (approximately 15–25 g each) will generally be considered overcrowded.

- Large cages containing more than ten mice will generally be considered overcrowded.

Identification and Resolution of Overcrowded Cages

All cages identified as overcrowded will be labeled by DCM staff with a yellow overcrowding cage card. The card will include the date the cage was identified.

The investigator is responsible for monitoring animal density and reviewing the Animal Health Monitoring sheet at least once every seven days. Investigators are expected to promptly address overcrowding and any identified animal health concerns.

The timeframe for resolving overcrowding will depend on its severity:

- **Moderately overcrowded cages:** Investigators will have seven days to separate animals in moderately overcrowded cages. If the lab does not attend and split the cages within this seven-day time frame, DCM staff will split the cage and charge for the cost associated with doing so.

- **Severely ("grossly") overcrowded cages:** Cages containing more multiple litters in a small cage will be separated immediately by DCM staff, without a waiting period and with out prior notification of the lab. Should this occur, a charge for the cost associated with splitting the cage will be incurred by the lab.

When DCM staff perform animal separations, mice will be separated by sex to discontinue further breeding.

Note: a charge will be assessed to the PI for each cage requiring separation by DCM staff.

Cage Card Requirements Following Separation

Yellow overcrowding cage cards must remain on cages until the animals have been appropriately separated.

When DCM staff perform separations, newly established cages will be identified with temporary blue cage cards to facilitate tracking of the separation. The investigator is responsible for replacing these temporary blue cards with standard cage cards.

Care and Receipt of Imported Rodents (Vendor Sourced)

Incoming animals from approved vendors can be admitted to existing colonies once the animals have been examined for signs of physical injury or disease by the investigator and/or an Animal Care Technician. The animals can only go into an assigned space.

- Upon arrival, a DCM staff member will check the animal crate(s) for any defects of concern. Depending on the facility and type/source of animal, a pre-arranged agreement exists between the Area Supervisor and the investigator as to who will unpack the

animals within an assigned housing room, e.g., some investigators receiving rodents, birds and frogs from approved vendors may be responsible for their own animals. Consult with the Area Supervisor beforehand about this and how you will be notified when your animals have arrived.

- After putting animals into a clean cage or tank with food and water, the shipping crate must be rechecked for animals that may be hidden. The empty crate is then taken to the dirty cage wash area of the facility.
- Whenever DCM unpacks animals, the research lab is charged a fee.
- Acclimation periods will apply to newly arrived animals, as warranted.

Husbandry for Different Animal Species (In Brief)

The following sections highlight husbandry practices that might impact a researcher, especially if the researcher is responsible for providing husbandry for the animals. In-depth information is available from the relevant Area Supervisor and/or veterinary staff.

Each cage must have a cage card with PI, protocol number, account number, contact person's name and cell phone number, and the strain and source of the animals.

Mice

- SPF (specific-pathogen free) mice housed in static microisolator caging. Cages have hardwood chip or BioFresh bedding and a nestlet. Singly housed mice also receive Enviro-Dri shredded paper nesting material. **Cage bottoms are changed at least once each week (twice if housing three or more adult mice).** Cages are spot checked daily for excessively dirty cages. Mice are transferred by gently grasping the mouse with forceps at the base of the tail or the scruff of the neck; forceps are sanitized with disinfectant between cages. Food and water are provided ad libitum unless an approved CAC protocol states otherwise. All food is discarded and replaced with fresh food once per month. Water bottles are replaced with clean ones at least once each week.

- SPF mice housed in individually ventilated caging. As above except cages on auto-water have lixits replaced with clean ones on a monthly basis. Cage bottoms are changed at least every two weeks (once per week when housing four or more adult mice).

- SPF mice housed in REX microisolator caging. As above except cages are lined with corn cob bedding and the entire cage unit is autoclaved before use. Cages must be marked by the researcher with salmon-colored "REX" cage cards. Cage units are changed at least once a week within the housing room or cubicle. Autoclavable rodent chow (Prolab 3500 or 5021 Autoclavable Rodent Chow) is added as part of the cage unit. Water bottles are autoclaved.

- SPF mice housed in SCID microisolator caging. As above for "REX" cages except all cage/animal handling must occur in a positive air flow change station or a biological

safety cabinet. Cages must be marked by the researcher with cream-colored “SCID” cage cards

Rats

- Similar to mice in static microisolator caging except a larger cage is used that has a height of 8” (versus 6” high for large mice cages).

Rabbits

- Rabbits are housed in plastic cages (change at least every two weeks) with grid floors suspended over excreta pans (change twice a week or as needed). A measured amount of high fiber rabbit chow is fed once daily, and timothy hay is fed several times weekly. Water is provided ad libitum through the automatic watering system (check lixits daily) and/or by bottle or water bowl (change daily).

Swine

- Swine are singly (if justified) or group housed in enclosed steel pens. Each pen is cleaned on a daily basis, and is sanitized biweekly. Chow is fed twice daily and water is provided ad libitum by lixit (check daily) or bucket (wash once a week).

Nonhuman Primates

- Discuss with DCM veterinary and facility staff.

Zebrafish

- Research labs with zebrafish colonies housed outside DCM animal facilities must submit husbandry SOPs to DCM (a template is available from DCM); information to be covered includes procedures for feeding, maintenance of room and tank sanitation, water quality, and monitoring of colony health. ***The updated husbandry SOP must be found within the animal holding room, either posted or in a central location***. A separate disaster plan must be laminated and posted in the housing area (a template can be provided). Social housing of fish should be the default, and single housing of fish should only be due to health reasons or approved to meet research goals. Enrichment should be provided to animals, though social housing is the default enrichment of fish housed at MIT

Finches

- Small breeding cages can house four adults or a breeding pair and offspring as long as the cage is not overcrowded; large flight cages can hold up to 20 compatible birds. Each cage is lined with cage board (changed at least three times a week), cuttlebones and perches are required for enrichment, bowls with seed (replaced three times a week or more often if needed) and water sippers must be available at all times. Cages must be changed and sanitized every 8 weeks.

Social Housing and Other Environmental Enrichment for Animals

Social housing in social species is the preeminent means of providing environmental enrichment. All rodents, birds, fish, frogs and nonhuman primates are housed as pairs or in larger groups, as cage size permits, unless they are separated due to **incompatibility** or because experimental paradigms prohibit co-housing. Singly housed mice and rats require a "Single Housing" card.

For social animals, social housing is the default for compatible individuals (health status, gender, behavior, experimental use) as described in the *Guide for the Care and Use of Laboratory Animals (Guide).* Exemptions may be based on clinical assessment by a veterinarian and/or their designee, attrition due to experimental manipulations or as approved in the CAC protocol, with provided scientific justification.

Environmental enrichment is designed to enhance animal well-being by providing sensory and motor stimulation, allowing the expression of species-specific behavior, and providing some control over its environment. DCM has developed standardized practices for enrichment of various species, which are described below. These standard practices may not be followed based on the scientific objectives of individual experiments. With the exception of social housing, formal exemption through the CAC is not required; instead, exemptions are managed by communication between the investigator, DCM's veterinary staff, and the Area Supervisor.

Note that radios may be placed by investigators at a low volume to produce white noise with the goal of reducing stress potentially associated with extraneous noise in the animal facility. Nesting material/manipulanda/human contact/food treats consistent with sound management and experimental use practices.

Species	Nesting	Manipulanda	Dietary	Cohort contact/exercise	Human Contact	Other
Fish			Live brine shrimp	Standard to group house		Substrate, plastic plants or "grass"
Frogs		Ramps (Rana) PVC tubes or floats (Xenopus) Spider wood perches and plants (litoria)	Crickets *(Rana and litoria)* Super worms *(litoria)*	Standard to group house		
Axolotls		PVC tubes		Standard to single house. Larvae are group housed		
Gerbils	Eco- Bedding or nestlets, nest box	PVC tubes, autoclavable un-treated wooden chew blocks or		Standard to group house		Dust bath

		manzanita				
Finches	Nesting box (breeding birds)	Cuttlebone; variety of nesting material, swings	Hard boiled eggs with High Potency Mash, fresh produce	Standard to group house		Variety of perches; water baths twice weekly
Hamsters	Eco- Bedding or nestlets	PVC tubes, autoclavable un-treated wooden chew blocks or manzanita		Standard to single house		
Macaques		Variety of in cage and hanging toys, mirrors, forage devices	Fresh produce, forage mix	Standard to pair house	Positive interaction with ACTs at least 2x/day	Videos offered on a rotating basis. Manzanita wood provided for single housed animals.
Marmosets	Hammock, nest box	Variety of in cage and hanging toys, mirrors, manzanita branches	Fresh produce	Standard to group or pair house	Positive interaction with ACTs at least 2x/day	Foraging devices provided for single housed animals, and on a rotating basis for all animals
Mice	Eco-Bedding or nestlets	PVC tubes and/or plastic igloo as needed		Standard to group house		
Rabbits	Plastic hutches, and nest boxes for breeding animals	Variety of in cage and hanging toys (dumbbells, jingle balls, cyclone chews, hanging rattles,etc) autoclavable un-treated wood blocks	Hay, NutraBlocks, PRIMA-Treats, Veggie-Chips, B-Wholesome Treats, fresh produce	Standard to singly house with minimum of 30 minutes of individual exercise once a week in a playpen		Social housing and play is encouraged for compatible animals
Rats	Eco-Bedding or nestlets	Nylabones (unless exempt), dumbbells, PVC tubes		Standard to group house		
Swine	Pine wood shavings when housed in non-elevated pens	Hanging and floor toys; foraging devices	Fresh produce	Group housed when possible based on size/source/use/compatibility. Limited contact, visual, olfactory, and/or auditory access with cohorts if possible, mirror if housed alone.	Minimum of 5 minutes 1X per day with ACT	Radio w/classical music or nature sounds offered on rotating basis

Standard Care Provided by DCM

Husbandry and animal welfare are integral components of the DCM program for animal care. The goal of our husbandry program is to ensure that the highest standard of animal welfare and

care are maintained. Special services that are provided through our animal husbandry staff include health monitoring, separation and/or weaning, euthanasia, shipping assistance, and administration of medications (as prescribed by the veterinary staff).
The following are standard care operations performed by DCM at a frequency detailed on room door sheets; consult the appropriate Area Supervisor with any questions:

- Water and feed all animals a standardized commercial feed
- Change all cages
- General cleaning of animal rooms; rooms swept/mopped and walls washed at the appropriate frequency
- Monitor and report the health of animals requiring veterinary attention to DCM veterinarians and researchers.

Researcher Responsibilities for Animals

All researchers working with animals are responsible for the following:

- Ensuring all animal procedures are pre-approved by the CAC.
- Knowing and following procedures described in CAC animal protocol.
- Adhering to all guidelines posted on holding room doors.
- Assuring the technical skill of personnel handling animals. Requests for technical training should be made to dcmtraining@mit.edu.
- **Monitoring health status of animals *(*including fish*)* at least once every 7 days and notifying DCM personnel if problems occur.** Looking at the "Animal Health Monitoring" sheet posted on the animal room door will aid the investigator in this process.
- Fish in core facilities (Koch/WIBR) monitored by facility and veterinary staff that experience morbidity/mortality should be noted on the animal health monitoring sheets located in the animal holding room.
- Information about export and import of zebrafish embryo and larvae should be recorded in the export/import log sheet in the zebrafish core facilities (Koch/WIBR).
- Responding quickly to veterinary recommendations.
- Properly documenting any special food or water formulations. Animal feed bowls and utensils used with special diets must be emptied and rinsed before being washed by DCM staff.
- Providing proper housing of rodents in the animal facility on day of arrival. DCM offers an unpacking service (a fee applies), which can be arranged through the Area Supervisor. **Animals cannot be left in delivery crates overnight!**
- Having accurate contact and identification information available for all animals (PI, protocol number, account number; contact person's name, cell phone numbers, email address in case of an emergency).

- Recording any changes in the animal population on the room census sheet.
- Taking precautions for personnel safety.

"Animal Health Monitoring" Sheets and Health Checks

Health check cards are always placed by the Animal Care Technician when they observe a sick animal. Investigators can also place health check cards which will initiate veterinary assessment of the animal. The date, observer, and problem will be noted on the card. Health check card information must be recorded on the "Animal Health Monitoring" sheet that is posted on the animal room door.

The veterinary staff makes daily rounds to evaluate any animals with a health check card. They will assess the problem and make comments/recommendations on the card. The card has room for investigator comments or action; provide acknowledgment that you have seen the card and what action will be taken. Once the card has been placed, animals are monitored at least once weekly. **Under no circumstance should investigators remove green health check cards until all animals within the same cage (rodents, birds, etc.) have been euthanized; however, DCM staff may remove the card if an assessment determines the original problem has been eliminated. The health check card can be moved behind other cage cards when the health issue is initially resolved.**

Anesthesia Machines for Rodents (IMPAC6, COMPAC5, VI)

DCM has several types of rodent anesthesia machines that are available for use by trained researchers.

Following is an overview for machine use:

- Isoflurane use must be described in a CAC-approved protocol.
- **Researchers must undergo training on machine use, which occurs during a basic rodent handling session.**
- Directions for anesthesia machine use are posted near each machine.
    - Isoflurane levels
    - Oxygen tank levels
    - Scavenging (weigh f/air canister)
    - Heating source
    - Appropriate animal density per chamber
- Complete billing sheet.
- Turn off machine, replenish isoflurane, clean machine chambers with Peroxigard wipes, change the oxygen tank if empty, and clean cart.
- Some mouse procedure rooms have anesthesia machines that use oxygen concentrators rather than oxygen tanks. Instructions for these units are posted near each unit. NOTE: The flowmeter is pre-set to the appropriate level and should not be touched.

Containment Areas/Using Hazardous Materials in Animals

Hazardous Material (hazmat)

Hazardous materials used in animals encompass a wide range of agents or substances. Biological hazards, chemical hazards or others are of concern as it, or its metabolites may pose a risk to humans and/or other animals (e.g., husbandry staff, veterinary staff). It is of utmost importance that the use of any hazard is approved by the CAC, notification to DCM about its use is provided prior to instillation in any animal, and proper handling, signage and other communication is in place.

Examples of hazmats used in animals at MIT/WIBR are:

- Biological hazmats (need a Biological project registration number):
    - Primary human cells
    - Rodent cell lines
    - Established human cells (require testing for murine pathogens)
    - Viral vectors (lentivirus, adenovirus, AAV, retrovirus, HSV Measles virus, influenza virus, others)
    - Friends Murine Leukemia virus (risk to other mice, not humans)
- Chemical hazmats
    - All nanoparticle/lipidoid formulations
    - BRDU, cisplatin, Streptozotocin, tamoxifen, etc.
- Radioactive hazmats (need Radiation Protection Program (RPP) authorization number for PI):
    - I125, Na24, K42, H3, C14, S35; including isotopes for PET imaging
    - Note that Cesium irradiation must be described in a CAC protocol and be separately approved by RPP, but irradiated animals are not hazardous to humans.

Containment Areas

Containment Areas (CAs) are designed to allow segregation of animals that receive hazardous materials (hazmats) and provide safe dosing/handling conditions within a ducted BSC or chemical fume hood. **No one may enter a CA without training first from DCM's Hazards Coordinator or designee. An established researcher can NEVER have a colleague "shadow" him/her into a CA.**

Obtaining Permission to Use Hazmats

Materials must be identified by the PI as part of the Animal Care and Use Protocol submitted to the CAC. EHS actively reviews animal care and use protocols and members of MIT Health Occupational Health Services program also serve as protocol reviewers for the CAC. Should the material(s) be approved by the CAC, a Risk Assessment Form (RAF) is completed. The RAF is a comprehensive document which is used to capture all aspects of proposed hazards used in

animals. It serves to communicate all aspects of a hazard which is proposed to be used, inclusive of the hazard(s) themselves, the route, the dose, details on housing, waste collection and handling and PPE precautions and decontamination requirements. **Hazard use cannot start until study personnel have discussed how many animals will be used, the study timeline, and the availability of dedicated (containment) space with the Animal Resources Manager**. In addition, the study personnel must receive an orientation in the animal facility on the use of specific hazards by the DCM Hazards Coordinator or designee.

Training of Hazmat Use in Animals

When experiments using a hazmat are to begin, **researchers must request and receive training in the animal facility about specific safety guidelines from the DCM Hazards Coordinator for that specific hazard use.** This training includes review of the pertinent items from the RAF form and ensuring that all persons handling the hazardous material are included on the protocol and apprised of the precautions associated with the hazard. The RAF is maintained in the area associated with the hazard. Note that no personnel can enter the containment areas without specific training from the DCM Hazards Coordinator, and there is no shadowing of unapproved personnel.

Responsibility of Researchers – Husbandry of Animals

Depending on the hazard, researchers will be responsible for handling animals for a certain period post-dosing, which is called the "risk" period. Any handling of animals or cages must take place in a ducted BSC or chemical fume hood during this time. For many chemical hazmats, "risk" periods run about three days (representing when the animal may be excreting the chemical or its metabolites), however EHS provides this information during the approval process of the hazmat, and is subject to change, based on the risk-assessment of the animal, containment and personnel. This is indicated in the RAF. After the risk period has ended, researchers are expected to change their cages into clean caging, thus, lowering the risk to DCM staff members. Disposal of contaminated bedding must be according to MIT/WIBR'S EHS directives. Most CAs are comprised of isolation cubicles which have negative air flow relative to the corridor; procedure/surgery areas are also available

In the case of prolonged dosing of the hazmat or if the hazmat has a long "risk" period (>4d), the researcher must provide If a hazmat is dosed often or has a long "risk" period (over four days), the researcher is responsible for providing animal husbandry (i.e. cage change, replenishment of food and water) for the duration of this time. The researcher must complete the **"Researcher-to-Provide-Husbandry" log sheet as indicated on the RAF. Husbandry specific to the species is articulated in the RAF and by the Hazards Coordinator. Note that husbandry may be required by research staff every day, with a complete cage change on Friday**. It is critical that there is documentation to ensure that animals are being evaluated consistent with DCM, CAC and EHS's expectation, and these logs will be audited and reviewed by DCM and the CAC to ensure compliance.

Husbandry entails checking the animals according to the RAF. This will be reviewed by the DCM Hazards Coordinator upon training.

Note: If your CAC protocol describes daily monitoring of animals, you are expected to come in on weekends to check on their welfare, regardless of a cage change on Friday.

Handling of Contaminated Carcasses/Bedding/Cage Equipment

During the "risk" period for a given hazmat, carcasses, bedding and cages are considered contaminated and must be placed in separate biohazard bags. The biohazard bag containing the carcass(es) is placed by the investigator into the nearest refrigerator labeled for this purpose.

Biohazard bags containing contaminated bedding and cage equipment are placed back in the cubicle or on a cart, depending on the CA. Facility staff will remove contaminated bedding and caging from this spot.

If a hazmat is dosed in a research lab, e.g., urethane for terminal anesthesia, the lab returns carcasses, bedding and cages in biohazard bags back to the animal facility for disposal or washing, as above.

Additional Expectations of Working with Hazmats in Animals are:

- DCM and research personnel are not put at risk, carcasses and bedding are disposed of properly, and adequate decontamination of non-disposable equipment is carried out.
- If research personnel are required to provide husbandry for part or all of the study, husbandry log sheets are filled out to provide documentation of the same.
- A relevant magnetic label is placed on the cubicle door or in the room when the hazmat is used in a Containment Area within an animal facility.
- Hazmats involving animals that are used in research labs, e.g., urethane, are handled appropriately.
- Additional gowning and training are required to enter a CA, work surfaces must be disinfected before and after use, and regular mouse holding rooms cannot be entered after being in a CA without a shower and change of clothes.

Euthanasia, Carcass Disposal and Euthanex Systems

Euthanasia

An "Animal Termination Form" must be completed when non-rodent mammals are euthanized (obtain from DCM veterinary staff).

Investigators and their staff may euthanize animals if personnel are trained and qualified; methods used must match those described in the approved CAC protocol. Animals are not to be euthanized in the animal room itself unless the room is under quarantine; the time that other animals are kept in the same area should be minimized. Instructions for CO_2 euthanasia are posted in procedure and necropsy rooms and must also be posted in your lab if you have CAC

permission to euthanize by CO_2 outside of DCM animal facilities.

Researchers who wish to donate their animals to DCM's Tissue Harvest program after their experiments are complete should email dcmvettechs@mit.edu.

Carcass Disposal

Rodents or small birds found dead by DCM personnel are placed in a plastic bag, labeled with date, investigator name, and room number, and stored in a designated refrigerator located in each animal facility. The cage is marked with a bright pink sticker that has the date when the dead animal was removed. All animals found dead will be noted on the "Animal Health Monitoring" sheet attached to each animal room door.

Investigators should complete an "Animal Termination Form" for all unexpected deaths of non-rodent species. **Investigators must immediately report unexpected deaths of non-rodent species to the veterinary staff in person or by phone.** Deceased nonhuman primates must be submitted to the DCM Comparative Pathology Laboratory for necropsy. This service is performed as part of DCM's tuberculosis surveillance program for nonhuman primates.

Uncontaminated carcasses and animal waste should be discarded in sealed plastic bags and placed in designated refrigerators of each animal facility. Rabbits and larger animals should be double bagged.

Contaminated carcasses and bedding (e.g., with carcinogens or infectious agents) must be placed in biohazard bags for incineration. Contaminated waste must be discarded according to procedures arranged through the appropriate program, the DCM Hazards Coordinator, and the Animal Resource Manager. Each hazardous material project involving animals must be associated with a **posted "bulleted" sheet** and "DCM Required Protection/Control Required for Hazard Use in Animals" form that details proper methods for contaminated carcasses and waste disposal.

Euthanex Systems

Placement of magenta **"To Euthanize by DCM"** cards by the investigator is sufficient notice for mouse/rat euthanasia requests**.** Animals in overcrowded cages, or that are weaning age or older, must be separated into multiple cages before placing "To Euthanize by DCM" cards on the cages. **All fields on this card must be filled out by the investigator before DCM will euthanize the cages of animals.** The labs should subtract these cages from their census sheets.

Euthanasia will be done in a Euthanex machine operated by trained DCM staff. This machine uses warm CO2 to anesthetize animals before euthanizing them. The method creates a more uniform, less stressful way to euthanize the animals.

All cages set for euthanasia must house animals that are sustainable on their own for multiple days:

- Animals must be clinically healthy

- Animals must have access to food and water
- Pups must not be left without a mother

Euthanex machines are not used with animals having active health check cards; instead, lab members must euthanize these animals themselves, e.g., using CO_2 in a procedure or necropsy space.

HVAC/Other Monitoring of Animal Facilities

A variety of monitoring systems are employed to monitor and control the environment in the animal facilities. The Building Monitoring System (BMS), which comprises a group of sensors, control devices, communications network and computer system, is operated by MIT's Physical Plant to monitor and control temperature and humidity in the animal facilities of MIT. BMS cycles the operation of HVAC ("heating, ventilation, and air conditioning") equipment in accordance with a system prescribed by DCM to provide optimum environmental conditions. WIBR has its own computer-controlled HVAC monitoring and control system as well as its own maintenance personnel. Each animal room also has a temperature and humidity display.

When detectors sense values outside of programmed limits, an alarm is triggered at Physical Plant's Operations Center and immediate action is taken. Physical Plant sends maintenance personnel to investigate the cause for the alarm and take corrective action. In addition to the BMS, "Hi/Low" thermometers are placed in all animal rooms and monitored daily.

Animal facilities and areas occupied by humans are ventilated separately. The desired positive or negative pressure in animal rooms and access corridors is accomplished by the interplay of supply and exhaust air velocities, with the net result being 10 or more changes per hour of filtered, un-recirculated and conditioned air in the animal facility. Particulate and toxic aerosols are removed from the air by the use of roughing and HEPA filters.

Physical Plant has established maintenance programs to ensure all filters for the HVAC systems are monitored and changed according to schedule. The Supervisor for Preventive Maintenance of Physical Plant maintains written documentation (work orders and signed receipts) of filter changes. MIT/WIBR's EHS Offices monitors the efficiency of the HEPA filters and ensures that air flows are checked quarterly and changed when required. The Industrial Hygiene Program staff of MIT/WIBR's EHS maintains records of air flow checks and dates of HEPA filter changes.

Vermin Control

Vermin control in all the animal facilities is directed primarily at cockroaches, silverfish, drain flies and rodents (wild or escaped laboratory rodents). Aside from obvious aesthetic problems, vermin can serve as mechanical or biological vectors for animal diseases. Rodents cause extensive building damage including chewing of electrical wires. Wild rodents may transmit infectious diseases to research animals.

- Control of insects: An ongoing surveillance program has been established by DCM's

Facilities Coordinator and a consulting entomologist; when and where needed, employment of "sticky" bug traps (not to be placed in rodent housing room floors), boric acid powder, PT-240 aerosol, squeeze bulb dusters and liquid bleach are employed. Drain flies are controlled by pouring liquid bleach down drains. No volatile insecticides are used in animal housing areas.

- Elimination of breeding sites for insects: This is a key element of insect control. Mops and brooms are suspended from the floor; food and bedding are maintained on pallets; waste containers and food bins are on castors; and shipping boxes are disposed of daily to facilitate the elimination of breeding sites.

- Control of rodents: Have-A-Heart and/or Ketch-All traps are used in animal rooms, feed storage areas, and other locations in the animal facilities. Trapped rodents are taken to 16- 859 for evaluation and euthanasia. If wild rodents are observed in or near the animal facilities, DCM's Animal Resource Managers or the Facilities Coordinator should be notified.

Investigators should minimize the amount of dirty caging in laboratory space as pheromones from animals and food will attract wild rodents.

Summary of Billing/Charges to Investigators

Investigators are asked to identify funding sources and accounts in Section 2 of their CAC protocol applications. Animal-related expenses should be charged only to the accounts listed in the relevant approved protocol; investigators are responsible for providing DCM with the appropriate account number(s).

Census and Per Diems

Census sheets, color-coded for each animal facility, are on clipboards that hang on the door of each animal room. A sheet exists for each PI's account and cost center. DCM Animal Care Technicians count individual animals each week and record numbers on the census sheets. **(Rats, mice, gerbils, and finches are counted by the cage; frogs are counted per holding tub at MIT and individually at WIBR. All other species are counted individually.)** DCM personnel collect census sheets on the last day of the month except when this falls on a weekend or holiday. In these cases, the census is done a day or so prior to the last day of the month. New sheets for the upcoming month are placed the same day the old census sheets are collected.

Investigators are expected to keep accurate counts of additions, reductions and transfers to their animal colonies on the appropriate census sheet(s). The census sheet is numbered for each day of the month and investigators are to record when additions or reductions occur. If investigators do not record additions or reductions, DCM assumes any additions occurred the day following the last count by Animal Technicians and any reductions occurred the day prior to the most recent count.

The information on the census sheet is entered into the billing program to generate the per diem charges for the month. These charges appear on the "Staff Account Bills". Investigators are to inform DCM immediately of any discrepancies in the animal census.

Per diem rates have been established for the various species of laboratory animals to recover the costs associated with the operation of the animal resource program at MIT/WIBR. Animal per diem rates is determined by using the principles established in the *Cost Analysis and Rate Setting Manual for Animal Resource Facilities* (NIH Publication, updated 2002). Per diem rates are subject to change according to operating costs.

DCM uses an integrated software program for cost accounting and the calculation of per diem rates for the animal resource program. All costs including labor, supplies, cages, and equipment associated with a particular species of animal are entered into the database program and distributed among those species. Those costs not directly associated with a particular species or cost center, but which support the animal resource program, e.g., administrative and professional salaries, general maintenance and cleaning supplies, computer systems, etc., are distributed among the cost centers according to the relative amount of space an animal occupies and the daily census of that animal. Overhead costs such as electricity, heat and building depreciation are not recovered through the per diem charges.

Monthly Bills

Principal Investigators (PIs) will receive a monthly "Staff Account Bills" report for the services and supplies provided by DCM for each account incurring charges. These reports are sent out the third week of each month and reflect charges for the previous month. The charges appear on the monthly "Detailed Transaction Report" issued by the Comptroller's Accounting Office of MIT and sent to all MIT/WIBR departments. The "Staff Account Bills" report is divided into three sections:

- Animal purchases. These include the cost of the animal, shipping containers, freight and/or transportation charges. Sometimes shipping costs appear a month or two after the cost of the animal has been billed.

- Per diem charges. These include the daily charge for maintaining animals. A care day is the unit used to determine the number of days DCM has housed an animal (or cage/tub, see above) for an investigator. The per diem is the daily rate charged for one day of care. The number of care days multiplied by the per diem rate yields the monthly charge. The billing report lists the total number of care days for each category of animal (referred to as a cost center on the billing) and the building and room where the animal(s) has been housed.

- Special services. These include charges for diagnostic testing, surgery supplies and services, quarantine testing, technical assistance, clinical treatments (e.g., gel food), fines for inadequate recordkeeping of food/water restricted animals and services such as separating overcrowded cages. This section of the project billing itemizes all

additional charges.

The “Staff Account Bills” report also lists the census activity for the month and notes the beginning and ending census counts, the number of animals received and the number of animals used or transferred. Administrative or fiscal officers are to inform DCM in writing of changes in account numbers; the funding information should also be updated in the CAC protocol.

Extra Charges to Researchers

- Unpacking animals
    - If DCM unpacks animals and puts them in cages, a task typically performed by the researcher for rodents from approved vendors (and frogs) in some facilities, a charge will be made to the lab’s account.

- Separating overcrowded cages
    - Researchers are expected to separate overcrowded cages within 7 days; otherwise, DCM staff will separate the cage and bill the research lab. Cages which are found grossly overcrowded, thus, a threat to the health and welfare of the animals within this cage, may be separated immediately, without notice to the lab. The lab is charged accordingly.
- Providing gel food/other treatments
    - Animals may need to receive treatments from DCM because of a clinical issue, e.g., debilitation or dehydration may be treated with “gel food”. Other animals may need to receive water containing an antibiotic (usually “Septra”) because of immunosuppression and/or infection. The cost of these treatments will be billed to the lab’s account.

- “Fines” for inadequate recordkeeping
    - The researcher is expected to maintain records appropriately when animals are food or water restricted; these records give assurance the animals are being well treated and can highlight an emerging clinical concern, e.g., weight loss beyond CAC protocol guidelines. When DCM staff has to contact the researcher because records are incomplete, a charge will be made to the lab’s account.

Chapter 7: Veterinary Care

Animal Health Concerns

Non-Emergencies, small animals/rodents

Smaller animals (e.g., rodents, birds, amphibians/reptiles) that need medical attention are identified at the cage level by a **green health check card**. This is typically placed by the animal care technician servicing the room, but can be placed by any member of DCM or research staff, should they have a concern regarding animal health. This health check is also recorded on an "Animal Health Monitoring" sheet posted on the door of the room. This informs the veterinary staff and researcher about the problem.

Researchers should monitor these door sheets and the green health check cards to familiarize themselves with clinical activities and any matters that might require their attention in animal rooms. The health check card serves as a record to document the health concern, the prescribed treatment and any other information which should be conveyed to the research staff. This health check card has space for documentation of treatment or actions taken by the researcher, or by the veterinary staff. The expectation is that the researcher will follow current DCM policies, which includes assessment of their animals once per week. Thus, **the researcher has 7 days to respond to recommendations made by the clinical staff. Current policy states that animals be observed by research staff at least once every seven days**. The veterinary staff will follow up on all health checks to ensure concerns have been resolved or are under control.

Debilitated animals must be euthanized or receive supportive care (fluids, nutritional supplement, etc.). Should animals be found by the veterinary staff to be in need of euthanasia, all attempts will be made to contact the lab directly. In these cases, DCM may request that the researcher euthanize the animal; however, should the researcher not be available, DCM reserves the right to euthanize any moribund or debilitated animals without researcher consultation (see below, Animal Health Emergencies). The veterinary staff will decide if supportive therapy is indicated for an individual case. Animals requiring support must be closely observed and support must be provided in a reliable and appropriate manner. Routine treatments or supportive care are provided by the researcher. If the researcher is unwilling or unable to provide these, DCM will do so and charge the research lab

Green health cards are NOT to be removed by the researcher; these must be kept on the cage until DCM veterinary staff removes them. If all animals in a cage are euthanized by the researcher, the card should be turned in to the animal care staff (can be left on shelf of rack where cage was located).

Euthanized animals are placed in carcass holding refrigerators in each facility and are identified by researcher name and date of death; after 48 hours, any unclaimed carcasses will be incinerated.

If you have concerns regarding the clinical findings and recommended treatment(s), you should contact the veterinary staff. Researcher/veterinary interaction is very important, and input from

researchers is encouraged.

Non-Emergencies, USDA-covered species / large animals

For large animals, inclusive of nonhuman primates, pigs, rabbits, or others, if a health concern or condition is noted, the veterinary staff should be contacted directly. A notation should occur in the animals record about the concerns found, and in conjunction with DCM veterinary services, after assessment, additional details should be included in the record / progress form. Any health concerns should be reported to the appropriate veterinary staff. For those species which may be group-housed (i.e., shrews, *Peromyscus* spp, etc.) health check concerns may be captured via cage-card as an alternative.

Note that in general, any large animal (USDA-covered species) which is euthanized due to illness or other health concerns should be submitted to the DCM Pathology group for complete diagnostic evaluation, including a gross necropsy and samples taken for histological analysis. Other animals may be submitted to the pathology group on a case-by-case basis.

Animal Health Emergencies

If there is an animal health emergency, requiring immediate attention by the research or clinical staff, a red dot sticker will be placed on the "Animal Health Monitoring" sheet posted on the door of the room. Animals needing immediate medical attention may be identified by anyone in DCM or the research staff. However, when an emergency is identified, the clinical staff for the facility should be contacted immediately.

Animals requiring emergency attention will be examined as soon as possible and the researcher notified of the clinical assessment. If the researcher cannot be contacted and the situation requires immediate action, the clinical staff will proceed as necessary to ensure the well-being of the animal.

Methods to Contact DCM for Animal Health Concerns or Animal Health Emergencies

Note that lists of appropriate DCM personnel and their phone numbers are posted in the corridors of each facility.

- **Outside of normal "working hours" (before 9:00 am, after 5:00 pm), or on weekends/holidays, DCM clinical staff should be notified immediately.**
    - Weekend rodent Veterinary Technician (for rodent concerns)
    - PCS on call Veterinarian (617-504-8375) for any animal health concern
        - DCM's main office extension/answering service (617-253-1757 or x31757). NOTE: after-hour messages made to the 617-253-1757 number should be clearly identified as animal emergencies to the answering service. Non-emergency messages left at X31757 after hours are not

screened until the following business day.

Study Endpoints

- *Humane endpoints* are used to reduce the severity and/or duration of pain and distress experienced by an animal. Criteria that can be used to end studies *before* the onset of pain and distress are ultimately the ideal humane endpoints. Humane endpoints must be validated to determine that the criteria used as earlier endpoints will ensure that study objectives will be met even if the study is ended early.

- *Experimental endpoints* are defined as the points in time when the aims of an experimental study have been achieved and the study is concluded.

Study endpoints are inclusive of ***both*** experimental endpoints and humane endpoints. The study endpoints should be clearly defined in the CAC application. After the study endpoint has been met, the animal may be euthanized (i.e. for terminal tissue harvest). **Unless scientifically justified, humane endpoints must take precedence over experimental endpoints**. If pain or distress must occur as the result of research, scientific justification must be provided if it cannot be relieved by analgesics, anesthetics, euthanasia, or other techniques. **Death as an endpoint is unacceptable** unless strongly justified scientifically. Every attempt will be made to reach the researcher by phone/email with any serious health issue. If the researcher cannot be reached or does not respond to DCM requests for immediate euthanasia, DCM staff will proceed with euthanasia if warranted. To avoid loss of data from valuable experimental animals, it is in the researcher's interest to observe animals frequently (at least once a week), respond quickly to health check cards, and interact closely with the veterinary staff. Maintaining current contact information on cage cards is also required so veterinary staff can reach appropriate research personnel.

Colony Management (Mice and Rats)

DCM provides colony management support for routine breeding, weaning, and genotyping of mice and rats, as well as related services. DCM colony managers work together with researchers to facilitate efficient and humane use of genetically engineered mice (GEM) and rats at MIT/WIBR. The objective of colony management is **to house the smallest number of cages possible to achieve breeding and research goals.** This enhances animal welfare by minimizing the production of excess animals never used for research or breeding. In addition, maintaining the smallest rodent colony possible helps researchers reduce the per diems charged to their labs. Such efficiency requires knowledge of experimental needs, the reproductive history of a particular line, and routine and timely weaning and genotyping of animals produced.

To request DCM colony management service, obtain investigator training or ask questions regarding any aspect of rodent colony management, contact dcm-rodentvettechs@mit.edu.

Guidelines for Setting-Up Breeding Cages

There are different breeding schemes possible depending on colony goals and numbers of animals available for breeding:

- In a **monogamous pair** scheme, a single male and single female are chosen and placed together for the duration of their breeding life. This method is effective if the investigator needs to know the exact parentage of the pups. The postpartum estrus of the female is used to maximize the number of litters per female. This is a less efficient use of space and requires more males than other methods.

- **Harem or polygamous breeding** consists of one male and two or more females. This is the most economical method of rodent breeding, resulting in the largest number of young from the smallest number of breeding animals. In this breeding scheme, the postpartum estrus is taken advantage of, which maximizes the number of litters per female. Harem breeding is very effective to use with lines that are not good parents because all females in the cage will assist in caring for the young. Harem breeding should not be used if investigators need to know the exact lineage of pups. However, cages must be frequently assessed to assure that breeding harems do not result in chronic overcrowding situations and also to assure that new pups are not being injured by older pups close to weaning age. Identifying and removing pregnant females from harem breeding cages or frequent assessment and weaning of cages (2-3 times per week) allows maximization of the number of breeding females (increasing the number of pups produced) in the harem while avoiding overcrowding. This method is especially effective in mouse lines that produce small litters.

- Males and females can be housed separately and brought together for mating – a method termed **"hand breeding".** This method is commonly used by investigators that require timed pregnancies. Males and females are placed together in the evening and left together overnight. The females are checked for plugs first thing in the morning and removed from the males' cages. Plugged females are set aside and unplugged females can be set up with the stud males again that night. Note that a plug indicates that the male is breeding but does not guarantee his fertility. This method can be used if there are limited numbers of males in a colony. It is the quickest way to impregnate large numbers of females in a relatively short period of time. This scheme is also effective when males and females are aggressive towards each other or if males are known to kill pups.

- In case of breeder mortality, new females can be added to an existing breeding cage easily, but new males cannot without the risk of lost litters. Pregnant or nursing females will sometime abort or kill their pups if a strange male is added to the cage. This may be true even if a male is removed and later returned to the same cage. The sensitivity to strange males varies between strains and lines of mice, but mixing foreign males into an established breeding cage should be avoided whenever possible. A new male should never be introduced into a breeding cage that already contains a male.

- Mice have a postpartum estrus that allows them to become pregnant a day after the previous litter was born, so breeders which are continually co-housed may have litters every 22-25 days. Implantation of the embryos is often delayed for several days when the mother is putting energy into nursing a large litter. Age at weaning is important to clarify during experimental design. Requests to routinely wean mice at less than 21 days of age should be added to the research protocol and approved by the CAC.

Breeder Productivity

Breeding productivity should be monitored routinely and accurate records on productivity, generation number, and number of pups born and weaned are important. Date of birth, date of weaning and number of pups per litter should be recorded on the cage card and/or in a notebook or electronic file. Some research studies require an exact date of birth; this may require checking breeding cages for new pups several times a week. Cages with newborns younger than three days of age should not be disrupted as new mothers may be prone to cannibalize their young.

Unproductive or poor breeders are an inefficient use of research resources. Breeders with chronically small litters should be replaced, unless this is characteristic of the line. For example, mice on a C57BL/6 background are notoriously poor breeders and will frequently cannibalize their first litter. To optimize production, look at the recorded sizes of each litter on the cage card and determine if the breeding pair is producing smaller and smaller litters**. If litter size is decreasing, it is time to set up a new breeding pair.** Breeders that have not produced a litter after six weeks of cohabitation should be replaced. In addition, breeders that have been co-housed for longer than six weeks without producing pups should be euthanized unless the mice are difficult to replace. Breeders are usually retired at six months of age unless the line is particularly productive in old age (e.g., 129/SvEv mice). It is important to ensure that replacement breeders are productive before euthanizing the original breeders.

Many GEM mouse lines will reach sexual maturity at 5-6 weeks of age; however, initial mating at 6-8 weeks of age instead will maximize breeding productivity. Initiating breeding at too young of an age can decrease productivity as small females may be unable to carry large litters. Initiating breeding of females later than 6-8 weeks also decreases efficiency and productivity, and naïve females that have not been bred by the age of three months may not be successful breeders. Females that have been breeding from 6-8 weeks of age can continue to produce litters up to one year of age, although the number of pups and frequency of litters may decrease. Males have a longer reproductive span than females, although both decrease in breeding efficiency with age. In most GEM strains, sperm counts drop off in males after six months of age. C57BL/6 males are especially likely to lose fertility with age. However, it is easier to successfully breed an old male mouse (>6 months of age) with no prior breeding experience than to impregnate an older female mouse (>3 months of age) with no prior breeding experience.

Weaning (Mice)

Mice are typically weaned at 21-24 days of age. However, some strains and lines mature more slowly or may be more fragile. These pups may require additional days in the home cage to

nurse, grow and/or transition to solid rodent chow. This slow maturation needs to be balanced against the birth of subsequent litters that will need to compete for mother's milk; a result could be that the older litters do well at the expense of the newborns.

Newly weaned rodents should have food placed on the floor of the cage in addition to a full cage-lid hopper of feed. Newly weaned mice in cages supplied by automatic water should also have a water bottle to ensure hydration for at least the first week. **Always double check that the water bottle is in the correct position and not leaking.**

Mice should be separated by gender at weaning (the veterinary staff can explain how to tell the genders apart) and re-checked at six weeks of age to confirm gender. Weaning pups after approximately 4 weeks of age increases the likelihood of unexpected pregnancies. This may impact research/experiments which were planned, and yield overcrowded, noncompliant cage densities.

Co-Housing Male Mice

It is preferable to co-house males' mice from the same litter during the process of weaning. Fighting is likely when adult male mice are added to an established cage of other adult male mice. Aggression between males varies between strains. However, it may result in fatal injuries and/or injuries which render the male mouse unsuitable for studies or breeding, or require euthanasia. Male mice may be singly housed, if no other compatible conspecific can be identified. These cages must be identified by a "Single Housing" cage card and an appropriate justification included.

As males reach 8 weeks of age, they establish social groups with one dominant male. The dominant male will have higher testosterone and sperm production than other males in the cage. Due to this phenomenon, males removed from these cages and re-established as studs for breeding may need a week to become productive breeders. This is important when optimizing embryo productivity for harvest, but is likely of little significance in routine colony management.

Co-Housing Female Mice

Unfamiliar female mice can usually be added to an established cage of females of any age. Fighting, if it occurs, should be brief and minimal, except under unusual circumstances. Certain strains (e.g., BALB/c females) can be aggressive towards each other, and in general, is discouraged.

Adding a young female to an established breeding cage may result in an older male mouse to begin breeding again, if no litters have been generated for a period of time. However, breeding groups larger than 2 females:1 male or adding 1 female: group of male mice often will result in animal fighting and/or male exhaustion.

Cross-Fostering Pups (Mice)

Mouse pups <14 days of age can be cross-fostered if the dam is found to be deceased or if she must be euthanized due to a clinical reason. A foster female should be identified which has a litter approximately born approximately within 3-5 days of the date of birth of the pups to be cross-fostered. To increase acceptance into the existing litter, it is helpful to "scruff" the foster female and have her urinate on the orphan pups, then integrate these pups into the nest with the existing litter. If no female can be identified, the orphan pups should be euthanized as they are not able to defecate or urinate without stimulation from the dam at or before this age.

Identification of the pups may be done by performing small ear notches or tail tip amputations with approval from the veterinary staff, if pups are cross-fostered to a different strain.

Pups >14 days of age may survive without a mother, but require wet food supplementation and/or gel water on the floor of the cage to facilitate accessibility. Mice weaned or orphaned at 14-18 days of age should always have wet food placed on the floor of the cage in addition to a full cage-lid hopper of feed. Wet food must be replaced daily and a health assessment performed by the investigator until it is apparent that the weanlings are self-sufficient.

Any cage of mice weaned early or cross-fostered should be identified with a green health check card along with cross-listing the health check request on the "Animal Health Monitoring" sheet posted on the holding room door. Animals in poor health should be reported directly to the veterinary staff by email, phone, or in person.

Colony Maintenance (Mice)

The baseline for maintenance of a GEM strain is two small breeding cages and two cages of weanlings (one of each gender). This combination will sustain a line, even in the face of an unexpected problem with breeders. DCM often refers to this setup as a "maintenance colony"; it is usually reserved for a line that is not actively being used for experiments, but needs to be sustained for possible future use. Breeding productivity may vary some with hard-to-breed strains, and more breeding cages should be set up in these situations.

Alternate options depend on research needs, as the number of mice needed for a research experiment determines the number of breeding cages that need to be maintained. For example, a lab requiring only five mice a month with a specific genotype may be adequately supplied using just two or three breeding cages, whereas a lab requiring 50 mice a month will need to keep many more breeding cages in order to meet demands. Several factors regarding the desired mouse's genotype and phenotype must be taken into consideration when determining how many breeding cages to set up. These include gender, inheritance of alleles, number of alleles in the line, and zygosity of the potential breeder mice.

A resource for researchers to help determine the appropriate sizing of breeding colonies for a specific research project is: Breeding Colony Size Planning Worksheet (accessible via: https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-and-husbandry-support/colony-planning).

Remember the goal is to house the minimum number of mice on the shelf to achieve the experimental goal, and the challenge is to be flexible enough to meet the changing demands of the research.

Salvage of Low Production Lines

There are several methods to retrieve a line when a mouse strain becomes unproductive. The simplest method is to add young wildtype females to the breeding cages. This approach may help to stimulate both older males and females into productivity. Another approach is to split the established males and females from one breeding cage into two separate cages and then add 8-week-old mice of the opposite sex to each cage to stimulate breeding. Allow two months for litters. Other methods include *in vitro* fertilization and ovary transplant; these services are offered by DCM's transgenics program.

Surveillance Program for Rodent Health Monitoring

DCM maintains specific pathogen-free (SPF) rodent facilities. Rodent pathogens may have a multitude of effects on research, and often preclude mice or rats from being able to be accepted by collaborating institutions. Maintenance of this SPF status is critical as most infectious agents are known to affect their hosts in ways that impact research results and reliability (e.g., immune system parameters); in addition, decontamination efforts are difficult, expensive, and laborious.

To monitor that colonies remain free of specified pathogens, an active surveillance program is in place in all rodent facilities. Most murine pathogens cause subclinical infections; thus, sentinel animals are used to detect the presence of these agents and are tested on a routine basis using various, validated, diagnostic tests. For mice, DCM utilizes Swiss Webster/CD1 animals as sentinels. These mice are housed in large cages with fluorescent orange cage cards and are exposed to dirty bedding from colony mice in the room. The sentinels are sampled every two months and examined for endo- and ectoparasites, serum antibody to specific pathogens (such as mouse parvovirus, mouse hepatitis virus), respiratory and bacterial pathogens, as well as histological exam of major organs. PCR-based testing of fecal pellets, skin swabs, and/or other tissues is performed for immunocompromised mouse colonies and other colonies determined to be at higher risk of pathogenic organisms as needed. Investigators will be notified in the event of a positive result from any testing of a sentinel animal, with the possible exception of some opportunistic organisms. Similar surveillance programs are in place for other rodent species.

Preventive Health Programs for Large Animals

Preventive health programs are in place for other animals, including USDA-covered species. In general, this preventative health program encompasses planned, periodic physical exam evaluations of animals by a veterinarian. Based on the species and/or the nature of use, testing and/or diagnostic samples may be obtained during these exams. For more information, please speak to the area supervisor or facility veterinarian.

Chapter 8: Overview of Analgesia, Anesthesia and Other Drugs

General Information on Analgesics and their Side Effects:

- Opioids – are narcotic analgesics used for moderate to severe pain. They act through mu (μ), kappa (κ), and/or delta (δ) receptors in the central and peripheral nervous system. The degree of analgesia each affords will depend on whether the drug is an agonist, partial agonist, or antagonist; the dose given; and specific pharmacokinetics in a given species. Side effects of opioids include respiratory depression, bradycardia, hypotension, and gastrointestinal hypomotility. Most are given parenterally, but some can be given orally. The duration of effect depends on the species and can range from 2-12 hours; sustained-release formulations are available in some cases. **All opioids are controlled drugs.**
    - Examples: Buprenorphine, butorphanol, fentanyl
- Nonsteroidal anti-inflammatory drugs (NSAIDs) – have anti-inflammatory, analgesic, and antipyretic properties at peripheral and central sites. Side effects such as increased bleeding time, gastrointestinal ulceration, and renal papillary necrosis can occur with high doses or prolonged use, and in animals that are dehydrated or have underlying disease. Care must be exercised when giving NSAIDs during periods of low blood pressure (e.g., anesthesia) or dehydration; in these cases, concurrent fluid administration is required. Most drugs are available in oral or parenteral formulations, and the duration of effect is usually 12-24 hours.
    - Examples: Meloxicam, carprofen
- Local anesthetics – act on peripheral nerves to stop the transfer of pain to the central nervous system. There are several routes of administration and types of drugs. They can be given topically, via local infiltration, intravenously, or into intrapleural/epidural spaces. Side effects, including hypotension, dysrhythmias, seizures, and CNS depression. are usually associated with high doses or intravenous administration.
    - Examples: Lidocaine – rapid onset (1-2 minutes), short duration of action (0.5-2 hours); bupivacaine – slower onset (5-10 minutes), longer duration of action (4-12 hours), more cardiotoxic than lidocaine

Analgesia

Pain and distress that experimental manipulations may cause to animals must be considered and minimized by careful study design and the appropriate use of pain-relieving agents. Whenever a surgery is performed on an animal, the type of analgesic drug, dose, frequency, and duration of use will depend on the invasiveness of the procedure. Many laboratory animals are considered prey species in the wild, and they tend to suppress overt signs of pain to avoid predation. **Because of this behavior, researchers must assume that a procedure known to cause**

postoperative pain in humans will cause similar levels of pain in animals.

Depending on the surgical stimulus and the anticipated level of pain, clinical assessments of an animal are needed to assess response to analgesics so it can be determined whether more of a given drug is needed sooner than a prescribed timepoint. Clinical observations must also be performed when administering analgesics to determine if treatments can be discontinued.

Analgesics are generally accepted to work better when given prior to a painful stimulus, as pain is easier to prevent than to control once an animal is experiencing pain. Pre-emptive analgesia has the added benefit of decreasing the amount of anesthetic required, and consequently the potential side effects, in addition to allowing for a smoother recovery. It also prevents the phenomenon of 'wind-up', where rapid, acute pain augmentation can lead to pathologic pain. In many cases, non-pharmacologic methods to reduce pain are also indicated and beneficial.

Overall, questions about the appropriate use of an analgesic and its dosage must be discussed with the DCM veterinary staff, as there are many variables associated with pain management, including the possible impact of a particular drug on a proposed study. Veterinary consultation is also required for any changes to a plan for analgesia.

The analgesia plan must be included in the animal use protocol and approved by the CAC.

Estimating Severity of Pain Based on the Surgical Stimulus

The following guide offers information on how surgical or related procedures are categorized as causing mild, moderate, or severe pain; this information can assist in determining the appropriate analgesic drug(s) and the minimum duration of administration.

These are only guidelines so a researcher must be comfortable in assessing pain in animals and/or seek appropriate expertise from the DCM veterinary staff. See section on rodents for more specifics.

Tissue/Pain	Mild	Moderate	Severe
Skin/Integument	Small incision, superficial tumor implantation, subcutaneous implants, tattoos, biopsies	Large, uncomplicated incision; skin grafts. catheter or vascular access port placement,	Major soft tissue excision
Muscle/Tendon	Small intramuscular implants	Muscle or tendon incision, implants	Extensive excision/dissection of muscle/tendon
Bones/Joints	Intraarticular fluid removal or injection	Bone or bone marrow biopsy, external fixation of a fracture, simple osteotomy, joint surgery	Fracture/repair, amputation of limb or multiple digits, bone or bone marrow harvest
Nervous System		Trauma, minor craniotomy, CSF tap	Nerve entrapment, nerve ligation, major craniotomy with muscle dissection and bone removal, laminectomy, intracerebral electrode implantation

Thorax			Thoracotomy, rib resection
Viscera		Orchidectomy, vasectomy	Visceral torsion, distention, ischemia (especially myocardial)
Abdomen	Laparoscopic surgical approach	Laparotomy (no inflammation), small visceral resection, enterotomy,	Laparotomy with inflammation or larger visceral resection (nephrectomy, splenectomy, ovariectomy, hepatectomy), heterotopic organ transplantation
Eye		Eyelid surgery, subconjunctival implants	Intraocular implants or surgery that may cause glaucoma, uveitis, or corneal ulcers; enucleation, retinal implants
Ear	Ear bar placement	Surgery to external pinna	Ear canal surgery
Oral cavity		Tooth extraction, tracheostomy	
Miscellaneous		Thyroidectomy, hypophysectomy, thymectomy	

Multimodal Analgesia

Once a procedure has been categorized as mildly, moderately, or severely painful, researchers can decide whether one or more analgesics should be given. The administration of more than one class of analgesic, as part of a multimodal approach to control pain, has been shown to have several benefits. For example, NSAIDs and opioids target different pain pathways and therefore can address the multiple origins of a painful stimulus. In addition, many of these analgesic drugs act synergistically, and it is possible to reduce the amount of one or both of the drugs needed when they are combined, reducing any potential side effects. Furthermore, the drugs have different durations of action and so prevent "breakthrough pain" from occurring between periodic assessments of an animal's pain level. The following is provided as a guide only.

Pain severity	**Analgesic approach**
Mild	May not require drugs or only a single agent, e.g., local anesthetic, NSAID, or opioid
Moderate	Combine 1-2 types of drug, e.g., local anesthetic + NSAID
Severe	Combine 2-3 types of drug, e.g., opioid + NSAID + local anesthetic/epidural/continuous rate infusions

Duration of Analgesic Coverage

The duration of analgesic coverage should be as long as needed based on the periodic assessment of pain level. It is expected that animals will be observed at frequencies that allow appropriate administration of analgesics if pain is evident. The following table is a guide only.

Pain severity	Minimum duration of analgesic coverage
Mild	0-24 hours
Moderate	24-48 hours
Severe	48-72 hours

Anesthesia

Anesthesia means "without sensation". Injectable and inhalation anesthetics are available for all species, and there are advantages and disadvantages to each. The anesthetic plan should be discussed with a veterinarian prior to protocol submission if you have questions about what may be most appropriate for your research.

Injectable Anesthetic Agents

For injectable anesthetics, dosing based on weight is required and sometimes additional doses are necessary. Care must be taken not to overdose the animal. Recordkeeping, in addition to that required for medical records, may be required as certain injectable anesthetics are controlled drugs.

Some injectable anesthetic agents in laboratory animals include (**discuss these and other options further with the veterinary staff**):

- Avertin (Tribromoethanol): Preparation and administration is described below for mice.
- Ketamine: A dissociative anesthetic, is a poor muscle relaxant when used alone (causes "plastic rigidity"); a Schedule III drug.
- Pentobarbital: A longer-acting barbiturate, is a respiratory depressant in some species; a Schedule II drug. Currently pharmaceutical grade pentobarbital is prohibitively expensive and often unavailable. Non-pharmaceutical grade pentobarbital is not available through DCM pharmacies and requires a lab-owned DEA license to purchase. Pentobarbital-containing euthanasia solutions obtained through DCM (e.g. Fatal Plus) CANNOT be used for anesthesia, even for terminal surgeries. These products may only be used for euthanasia only, or as a part of the euthanasia process (i.e., to facilitate perfusion).
- Urethane: Only used for terminal procedures in small animals, good analgesic properties, a hazardous material (carcinogenic and hepatotoxic, Supplement I required).

- Xylazine: Causes sedation, analgesia and good muscle relaxation with minimal effect on the respiratory system; often given with ketamine. Not sufficient for surgical anesthesia when used alone. Important side effects include hypotension, bradycardia, and arrhythmias. Reversal agents are available.
- MS222: Used for sedation, immobilization, and anesthesia of fish and amphibians by adding to a holding tub with tank water, i.e., not injectable. MS-222 is currently available in a pharmaceutical grade formulation and is equivalent in cost to non- pharmaceutical grade formulations. MS-222 that is applied to animals should be pharmaceutical-grade. The only pharmaceutical grade formulation is sold by Western Chemical, Inc. This is a hazardous material (lung and skin irritant) that must be prepared using safety measures for humans (prepared in a chemical hood, respiratory protection worn) and be buffered before use.

Inhalation Anesthetic Agents

Inhalation anesthetics are often desirable due to ease of administration, quick recovery times, and ability to control depth of anesthesia more readily. However, specialized equipment and training are needed, and appropriate scavenging of waste gases and disposal of expired or unused anesthetic agents is required.

Isoflurane is the inhalational anesthetic most commonly used in laboratory animals. It has a pungent odor and is a potent respiratory depressant. Excess isoflurane must be disposed of as a liquid hazardous waste and NOT vented. The only time venting is acceptable is when there is only residual liquid in the bottle that needs to be removed before disposal/recycling of the bottle. Any expired or otherwise unused isoflurane should be tagged with a standard red tag, placed into the Satellite Accumulation Area (SAA) and a request for pick up submitted online. Sevoflurane is also available for large animals. **Note: Ether is highly flammable/explosive and its use is strictly forbidden.**

It is critical to ensure that oxygen is readily available throughout the entire procedure to maintain consistent anesthetic range by providing delivery of isoflurane to animals. Reference the table below for estimating the remaining oxygen level based on flow rate and PSI.

Estimating remaining available time in oxygen tanks:

Times for remaining oxygen in E cylinder				Time for remaining oxygen in H cylinder		
	Minutes remaining	Minutes remaining			Minutes remaining	Minutes remaining
PSI in tank	Flow rate of 1 L/min	Flow rate of 2 L/min		PSI in tank	Flow rate of 1 L/min	Flow rate of 2 L/min
2200	560	280		2200	6280	3140
2100	532	266		2100	5966	2983
2000	504	252		2000	5652	2826
1900	476	238		1900	5338	2669
1800	448	224		1800	5024	2512
1700	420	210		1700	4710	2355
1600	392	196		1600	4396	2198
1500	364	182		1500	4082	2041
1400	336	168		1400	3768	1884
1300	308	154		1300	3454	1727
1200	280	140		1200	3140	1570
1100	252	126		1100	2826	1413
1000	224	112		1000	2512	1256
900	196	98		900	2198	1099
800	168	84		800	1884	942
700	140	70		700	1570	785
650	126	63		650	1413	706.5
600	112	56		600	1256	628
550	98	49		550	1099	549.5
500	84	42		500	942	471
450	70	35		450	785	392.5
400	56	28		400	628	314
350	42	21		350	471	235.5
300	28	14		300	314	157
250	14	7		250	157	78.5
Replace by 200 PSI				Replace by 200 PSI		

Controlled Drugs – Storage and Security

DCM maintains controlled drugs in two locations, 46-7013 and E25-021, for internal use as well as for dispensing to researchers. Controlled substances are drugs or chemicals that have the potential to be addictive or habit forming. The U. S. Drug Enforcement Administration (DEA) has divided the controlled substances into 5 schedules (I-V) based on their potential to be habit forming and usefulness in medicine. **The purchase and use of controlled substances in**

Massachusetts requires registration with both the DEA and the Commonwealth of Massachusetts.

The following information is for researchers that obtain controlled substances (Schedule II–V) from DCM. DCM's DEA registration does allow for dispensation to non-registered PIs with approved CAC protocols. However, non-registered PIs may not distribute these materials to other laboratories or PIs. Any lab member that is handling, receiving, or requesting controlled drugs must complete the "controlled substance use in lab" training. **Labs should not receive drugs from other sources, including other labs or institutions, unless the PI has his or her own registration in place to accept that controlled substance. Contact MIT/WIBR's EHS for more information on receipt of controlled substances from a source other than DCM.**

Recordkeeping Requirements

Any researcher using controlled drugs must maintain a "Continuous Inventory of DEA Controlled Substances" form where the drugs are stored; these forms must be kept a minimum of 2 years from the date of record. A copy of this form must be given to the DCM dispensing pharmacy along with the empty bottle before additional drugs can be requested. Contact MIT/WIBR's EHS or DCM for a copy of the inventory form.

Security

All controlled drugs must be kept in a double locked enclosure. Schedule II controlled substances must be stored in a "double lock" drug safe or a lockbox that is securely fastened with a security cord within a locked cabinet. Researchers are permitted to keep schedules III-V in a securely locked, sturdy, immovable cabinet or safe. However, if any schedule II materials will be stored along with schedules III-V, a double lock security system must be in place. Contact MIT/WIBR's EHS for more drug storage information or to request a lockbox and security cable.

If there is a theft or loss of Controlled Substances, DCM and MIT/WIBR's EHS must be notified. A DEA form 106 must be submitted to the DEA and the State Administrator must be notified within 10 days of the discovery of the theft or loss. DEA form 106 can be found at: https://www.deadiversion.usdoj.gov/21cfr_reports/theft/theft-loss.html

Disposal of Controlled Drugs

Expired drugs (controlled or otherwise) must never be administered to animals. Expiration dates should be monitored, and expired controlled substances obtained from DCM should be returned to DCM for disposal (unneeded controlled drugs should also be returned to DCM).

MIT/WIBR's EHS may be contacted for assistance with disposal of controlled substances obtained under a PI's personal DEA license.

Additional Information

- Controlled drugs dispensed in a labeled syringe must be used on the day of drug pickup.
- If the drugs requested will not be used that day, they must be dispensed in a sterile vial labeled with the drug name, concentration, amount dispensed, date dispensed, dispensed by, and expiration date. Drugs dispensed in a sterile vial must be kept in a locked cabinet (double locked if Schedule I or II are stored), and each aliquot dispensed must be recorded on a "Continuous Inventory of DEA Controlled Substances" form.
- The "Continuous Inventory of DEA Controlled Substances" form must be kept in your research lab close to the drugs. This form will be reviewed by DCM staff and the empty vial returned to 46-7013 (X45444) or E25-021 (X33050), depending on which dispensing pharmacy was used, before new drugs can be obtained.
- You must be able to account for the total amount of controlled drugs that were dispensed to you. This entails completion of the "Continuous Inventory of DEA Controlled Substances" form for each drug dispensed and includes date, amount used, reason for use, amount remaining, and signature of the person administering the drug.
- All unused or expired controlled drugs must be returned to a DCM pharmacy.
- Prior to obtaining additional drugs, the empty bottle and a copy of the inventory log sheet must be given to the dispensing pharmacy.

Expired Drugs and Supplies

1. Once a month, all drugs and supplies with expiration dates should be checked and disposed of appropriately.
2. Expired drugs cannot be used in animals regardless of the procedure being performed.
3. Expired, non-controlled drugs can be kept in a bag labeled "EXPIRED - Do NOT Use" in a locked drawer or cabinet until they are:
    a. Put in the trash or down the sink if non-hazardous, e.g., outdated sterile water or saline, multiple-use fluid bags, OR
    b. Have picked up by MIT/WIBR's EHS as hazardous waste, e.g., isoflurane. A hazardous waste collection request can be made at https://ehs.mit.edu/regulated-waste-program/chemical-waste/
4. Expired controlled drugs must be returned to DCM or MIT/WIBR's EHS for disposal. Until disposal, expired controlled drugs should be clearly labeled "EXPIRED – Do NOT Use" and stored in the drug safe or lockbox but segregated from the current, in-use stock. These are destroyed by MIT/WIBR's EHS on a quarterly basis. For investigators with their own DEA license who have ordered drugs directly, MIT/WIBR's EHS should be contacted for pick up and disposal. To schedule MIT/WIBR's EHS to collect and destroy expired or unused controlled drugs, contact MIT/WIBR's EHS (X23477) and provide them with a list of expired drugs and volumes. MIT/WIBR's EHS will complete Form 41 detailing the drugs and amounts and will give a copy to file.
5. Expired supplies, such as suture material, can be used in terminal animal surgeries, e.g., for the training of surgeons. These should be segregated and marked "Expired, for terminal

surgical use only".

Fluid Therapy

Fluid therapy is advised for surgical patients to provide replacement of blood loss during a surgical procedure, as well as to prevent dehydration postoperatively when animals will not drink. For rodents, warm sterile 0.9% saline or Lactated Ringer's solution can be given subcutaneously or intraperitoneally at a rate of 20 ml/kg. For larger species, where intravenous access is possible, warm sterile 0.9% saline, Lactated Ringer's solution, or 5% dextrose in saline should be provided at a rate of 4 ml/kg/hr throughout the procedure.

Note that fluid bags stored for chronic use are effectively multi-dose vials. The use of multi-dose vials introduces the risk of contamination and has been linked to a variety of hospital-acquired infections. Therefore, fluid bags saved for multiple uses must be maintained with aseptic technique and require an expiration date of one month after initial penetration. Prior to each use, the injection port should be cleaned with sterile 70% alcohol and allowed to dry, and a new sterile needle and syringe used to draw up the fluids; never enter the bag with a needle and syringe that have been previously used. The expiration date of 28 days beyond first use must be written on all bags at initial penetration and the bag(s) discarded when the expiration date is reached. Regardless of expiration date, a fluid bag should be disposed of sooner if the sterility is in question. Storage of in-use fluid bags in a refrigerator (4°C) is recommended. Fluids containing dextrose should not be saved and used as multidose vials.

References

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3. Davis H, Jensen T, Johnson A, Knowles P, Meyer R, Rucinsky R, Shafford H. 2013 AAHA/AAFP fluid therapy guidelines for dogs and cats, J Am Anim Hosp Assoc 49, 2013, 149–159.

Paralytic Drugs

Federal regulations prohibit the use of paralytics in unanesthetized animals; therefore, use is restricted to balanced anesthesia techniques or non-surgical applications that have been scientifically justified to the CAC. See the CAC policy "Paralytic Drug Use" for more information.

Use of Antibiotics

Due to emerging drug-resistant bacteria, the use of antibiotics in perioperative and irradiated animals should be minimized. Antibiotic use must be approved by the CAC unless it is prescribed by the veterinary staff. Contact DCM veterinary staff for more information.

Non-Pharmaceutical Grade Compounds Use in Animals

Federal regulations require that compounds used in research animals, including those used in non-survival procedures and euthanasia, be pharmaceutical grade. A pharmaceutical grade compound is a drug, biologic, or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by a national or regional pharmacopeia, such as the United States Pharmacopeia-National Formulary (USPNF).

Many commonly used drugs are available as pharmaceutical grade preparations, including most anesthetics, analgesics, and antibiotics. Common vehicles such as saline, corn oil, and DMSO are also available in pharmaceutical/medical grade. Laboratory-grade reagents, such as phosphate buffered saline (PBS), should not be used for this purpose. Please see the CAC policy on "Non-Pharmaceutical Grade Compounds".

Chapter 10: Introduction to Surgery

Facilities, Services, Equipment and Billing

DCM operates two centralized surgical facilities in compliance with the Animal Welfare Regulations (AWRs) and the *Guide for the Care and Use of Laboratory Animals* (*Guide*, used by Public Health Service (PHS) Policy). Activities that involve surgery must include appropriate provision for pre-operative and post-operative care of the animals. All survival surgery in USDA-covered species must be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques.

Major operative procedures on animals, other than rodents and birds bred in captivity, will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions. Non-major operative procedures and all surgery on rodents and birds bred in captivity do not require a dedicated facility but must be performed using aseptic procedures.

In general, the surgical suite should contain the operating room, a surgical support room, a surgeon preparation area, an animal preparation area, and an area for animal recovery.

In contrast, major survival surgery on rodent species and birds need not be performed in a dedicated surgical facility but must be performed using aseptic technique.

Facilities

Dedicated facilities for major surgery procedures involving non-rodent mammalian species are located in 46NHP and E25. Access to these areas is restricted and requires veterinary staff approval. Operating rooms are available for use Monday through Friday, 9 am to 5 pm. Surgery at other times must be arranged in advance with the veterinary staff. F**riday surgeries are subject to the policy found below. Investigators should notify the surgical staff of any cancellations or delays.**

DCM Policy for Elective Surgery on Fridays: Elective surgery of an invasive nature (i.e., a major operative procedure) and surgeries which may be of a less invasive nature are in general, not scheduled on Fridays in either of DCM's centralized operating rooms. Extenuating circumstances can be discussed with the veterinarian of the pertinent DCM surgery facility and will be evaluated on a case-by-case basis. Emergency procedures and, in general, rodent surgery are exempt from this policy. Invasive procedures on rodents will be prohibited, however, when morbidity resulting from such procedures is expected.

Services

Each surgery facility is staffed by a surgical veterinarian and technician as well as other members of the clinical veterinary staff. These individuals are available for consultation and assistance in a variety of areas as described below.

- Scheduling surgery/procedures. The surgery technician schedules the surgical rooms on a

first come - first serve basis. Scheduling must be done a minimum of 2- 4 weeks in advance of the procedure. Please submit a completed request form to dcm-scheduling@mit.edu to schedule surgery or procedures or to reserve surgical space . Request forms are available on the DCM website: Large Animal Technical Services | MIT Division of Comparative Medicine

- Surgical supplies. Surgery technicians are available to prepare sterile packs and instrument trays for surgery. If equipment and supplies are needed, submit a request to the DCM surgical facility a minimum of 48 hours prior to surgery. For equipment which must undergo Ethylene Oxide sterilization, a minimum of 7 business days is required, due to the off-gassing requirements of some materials.
    - Instruments, gowns, towels and drapes may be limited and are provided based upon availability. Microsurgical, orthopedic and specialized instruments and equipment should be provided by investigators. Materials for steam sterilization should be presented to the surgery technician by noon of the day before the procedures. Materials for gas sterilization require at least two full working days prior to the day of surgery.

- Surgical room preparation. The surgery technician is responsible for surgical room preparation. Table drapes, circulating hot water heating pads, surgical microscopes, monitoring equipment, anesthesia carts and other equipment are provided as needed. The research team is expected to ensure the space is clean, neat and tidy after surgery, while the surgical technician will provide disinfection of the space.

- Surgery consultation. A surgical veterinarian is available for consultation on the design of experiments involving surgery, anesthesia, and diagnostic imaging. Experimental needs that cannot be met with available resources at MIT may be planned in collaboration with neighboring institutions.

- Surgery support. Surgical support is provided, as time allows, by the surgical veterinarian. Surgical support includes, but is not limited to, celiotomy, thoracotomy, craniotomy, device implantation, vascular access, and endoscopy. Arrangements should be made in advance with a surgery veterinarian by contacting dcm-scheduling@mit.edu

- Anesthesia. The surgical staff is available on a fee-for-service basis to induce, maintain, and monitor anesthesia in all species. Injectable and inhalant anesthetics are available. Investigators should make arrangements as far in advance as possible to coordinate staff schedules.

- Equipment. The surgical facilities maintain a large inventory of equipment for use by DCM and qualified investigators. Equipment inquiries should be addressed to the surgical staff at the time of requesting the procedure.

- Billing. Services and supplies are charged to investigators' accounts. A fee schedule is

available for review from the surgery technician. Charges are invoiced monthly and are separated into the categories “Services” and “Supplies”. These charges appear on the monthly “Staff Account Bills” report that DCM sends to investigators.

Surgery Wet Lab

The CAC and DCM require that all individuals who will be or are performing surgery (survival or non-survival) are trained and refreshed once every three years. First time surgeons and anyone involved in post-operative care of surgical animals at MIT/WI are expected to complete an initial ecourse and attend an in-person surgical wet lab. The refresher version of the ecourse must be completed every three years.

Multiple wet labs are held throughout each month and last approximately one and a half to two hours. Training includes animal preparation, aseptic technique, instrument preparation and use, tissue handling, wound closure and suturing, postoperative care and monitoring, and analgesia/anesthesia. Participants are expected to seek additional personalized training from senior lab members and/or DCM veterinary staff on an as-needed basis. For USDA-regulated species, veterinary staff provides individual training and oversight, and surgeries are to be performed only in DCM-managed or -approved surgical suites.

Each wet lab attendee will have the opportunity to perform a surgical procedure on a mouse, and further training can be provided as needed. The wet lab is intended to clarify and reinforce what is required when performing surgery on an animal. New surgeons must attend a wet lab session and receive training from a senior lab member proficient in the technique(s) before performing surgeries independently. Consistent with the Undergraduate/UROP policy, undergraduates must have direct supervision from their immediate supervisor during surgery.

A separate wet lab is required for surgeons working with zebra finches.

Aseptic Technique

Aseptic (meaning “free of disease-causing organisms”) technique requires strict adherence to practices in a number of different areas. These include surgical equipment and instruments, preparation of the patient, surgeon, and surgical procedure space.

Sterilization of Surgical Instruments

All surgical instruments, implantable devices, and pieces of equipment that will contact the surgical site or be implanted in the animal are to be sterilized, using one of the techniques described below. The methodology selected will depend on time considerations, specialized equipment available, and the composition of the material to be sterilized. Proper sterilization techniques must be followed for the particular method in order to obtain consistent results. Sterilization monitoring devices should be routinely used to validate sterilization technique

For rodent and non-mammalian species, instruments must be sterilized using an autoclave or other approved method prior to each day of surgery. An extra set of sterile instruments should

always be available as backup. When multiple surgeries are planned, the use of glass bead sterilizers and/or the use of additional packs are required. It is acceptable to operate on multiple rodents using a single instrument set as long as blood and tissue debris are removed between procedures and sterility is not compromised, i.e., instruments should be re-sterilized between animals using a hot bead sterilizer for a recommended maximum of 5 surgeries.

Soaking instruments in alcohol and/or flaming is not acceptable as a method of sterilization.

Surgical Pack Preparation and Storage:

All surgical supplies and equipment must be cleaned prior to sterilization in order to remove any organic material that may interfere with the sterilization process. Surgical instruments may be cleaned in an ultrasonic cleaner or by hand, using a stiff bristle brush and a moderately alkaline, low suds detergent. Deionized or distilled water is preferred for cleaning. Once dried after cleaning, surgical supplies should be wrapped in barrier materials according to the pack size, instrument use and potential storage time (detailed in the table below). Materials should be placed in the autoclave in a manner that allows steam access to all surfaces, **do not layer items on top of each other to allow for proper sterilization**. Wrapping techniques are such that the surgical packets can be opened easily without touching any of the sterilized equipment or instruments. It is recommended that for large surgical packs, a chemical monitor indicator be placed in the deepest portion of the pack to ensure complete sterilization.

Wrapping Material	Shelf-life*
Cotton muslin – 2 layers	2 days
Double wrapped cotton muslin (2 layers each)	3 weeks
Double wrapped nonwoven paper	6 months
Plastic-paper envelope	12 months
Inner paper wrap and outer paper-plastic envelope	24 months
Polypropylene peel pouches	12 months
Double-wrapped disposable barrier nonwoven drape	12 months

* Sterile packs should be stored in an environment where airflow, temperature and humidity are controlled. The storage area should be dry and out of direct sunlight, away from heat sources and free of dust. The preferred storage method of sterilized surgical packs is closed cabinets. Sterile packs should be labeled with expiration dates according to the table above.

Steam sterilization (Autoclave)

Steam sterilizers are the most common method of sterilization and kill microorganisms through coagulation and denaturing proteins by moist heat. Materials should be checked for autoclave compatibility prior to sterilization.

Sterilization cycles are autoclave specific, but generally the following cycles can be used:

Soft goods and large surgical packs	35 min 250° F (121° C), 23 psi
Standard surgical pack	30 min 250° F (121° C), 23 psi
Flash sterilization (metal instruments with no lumens only)	30 min 250° F (133° C), 22 psi

All instruments and soft goods should be allowed to dry for a minimum of 15 minutes and then cool following sterilization and before use. Personnel should be trained to use the autoclave either internally or by coordinating with the DCM pharmacy (dcm-pharm@mit.edu). When using autoclaves in DCM areas, place a loose chemical monitor on top of the packs to be sterilized. Once that load is finished, place the loose autoclave strip in DCM's autoclave records, which should be located next to the autoclave. A loose autoclave strip should be used in every load, as this is used for DCM's records of autoclave validation.

Ethylene oxide sterilization

Ethylene oxide is a low-temperature method of sterilization using ethylene oxide (EtO) gas causing alkylation of proteins and nucleic acids obstructing cell metabolism and reproduction. Ethylene oxide sterilization should be performed in automated sterilizer as ethylene oxide gas is highly toxic. , All products sterilized with ethylene oxide must be aerated for a period of time to allow diffusion of the gas from sterilized objects. Sterilization times depend on the type of material and the type of aeration (mechanical or passive); in general, 48 hours are sufficient. .

Ethylene oxide sterilization is ideal for implantable devices or fragile equipment when steam or heat may alter or destroy the component materials. Ethylene oxide sterilization should not be used on materials previously sterilized by irradiation and cannot be used on liquids. One ethylene oxide unit is located in E25 and may be used by contacting the DCM pharmacy (dcmpharm@mit.edu). **Please allow 72 hours for a response when making EtO requests. Plan to sterilize the items at least 1 week before needing them.**

Hydrogen peroxide gas plasma sterilization (Steris MD140X)

Hydrogen peroxide plasma sterilization inactivates microorganisms primarily by the combined use of hydrogen peroxide gas and the generation of free radicals (hydroxyl and hydroproxyl free radicals) during the plasma phase of the cycle. This system uses a low-temperature, fast cycle (30 minutes) to sterilize instruments and medical devices. No absorbable materials (cellulose, foam, or linen, wood), liquids, or powders can be placed in the Steris sterilizer. **Use of this sterilizer requires specific training,** which can be coordinated by contacting the DCM pharmacy (dcmpharm@mit.edu). Please allow 72 hours for a response when making Steris requests and do not wait to sterilize the items. Plan to sterilize the items at least 1 week before needing them.

Cold Sterilization

This refers to the process of soaking instruments in sterilizing chemical germicidal solutions; the ideal sterilizing agent is one which destroys all bacteria, bacterial spores, and viruses. Cold sterilization may only be accomplished by the utilization of glutaraldehyde compounds, available under the trade names Cidex and Sporicidin. As with all previously described sterilization procedures, instruments should be free of organic debris prior to placement in the sterilizing solution. Instruments sterilized in glutaraldehyde must be rinsed in sterile water prior to use on the animal. Cold sterilization fluids must be discarded as hazardous waste. Note that most of these solutions are only considered high level disinfectants after a soak of approximately 20 minutes, and a longer contact time (>24h) is required for sterilization. Follow the manufacturer instructions for the specific product.

Hot Bead Dry Sterilizers

These are designed to sterilize the tips of instruments and are largely used for surgeries in rodents and birds. Depending on the model, they may take about 20-30 minutes to heat up, reach over 233°C (450°F), and take 15-30 seconds to sterilize the instrument tip. Instruments should be cleaned of blood and tissue with sterile saline and sterile gauze, and dried prior to placing in a hot bead sterilizer. Repeated use and prolonged exposure to this temperature will dull or destroy delicate instruments. Instrument tips should be placed on a sterile field and allowed to cool prior to use. It is recommended that instruments should be re-sterilized between animals using a hot bead sterilizer for a recommended maximum of 10 surgeries. Follow instructions based on model of sterilizer.

Wound Closure: Suture Selection and Suture Patterns

The type of suture and suture patterns selected depends on the application, and the size of the suture and needle type is also important to consider.

A chart is provided below to assist in suture selection and suture patterns

Suture material: Suture materials function to hold tissue in apposition and carry all of the physiologic forces until wound healing progresses to the point that the tissue is healed enough to sustain the forces. Choice of suture material is determined by the tissue type, size of animal and potential healing time. Suture material is supplied with the needle attached and this is known as swaged. Skin sutures should be non-absorbable and monofilament, e.g., nylon, prolene, monofilament steel. Braided non-absorbable suture like silk should not be used in skin because of the potential for bacterial colonization within the interstices of the braid and the consequent potential for wound infection via capillary action. Suture materials must be sterile because they are a foreign material and may provide a substrate for bacterial proliferation. Skin sutures or clips should be removed in 7 to 14 days after adequate healing has occurred.

- Suture size. The larger the number followed by a "-0", the smaller the suture. If no "-0" follows a number, then the larger the number, the larger the suture, i.e., from smallest to largest is 8-0, 7-0, 6-0……..1-0, 0, 1, 2…

- Needle type. In general there are tapered needles and cutting needles. Tapered

needles are intended to penetrate peritoneum, abdominal viscera, and subcutaneous layers with minimal cutting of the tissue. Cutting needles are intended for more tough tissue, like skin, fascia, and ligaments. The shape of the needle also varies from straight to curved. In general, a 3/8 circle needle is sufficient for most procedures.

- Longevity of suture material. Absorbable suture disappears within a certain time (e.g., coated Vicryl absorbs in 56-70 days, PDS II absorbs in 180-210 days, nylon and Prolene do not get absorbed). Most often it is not necessary to have suture that lasts indefinitely, and absorbable suture material is fine. For skin, nylon suture is preferred due to cost.

- Monofilament versus Braided suture. These refer to the construction of the suture. Monofilament sutures are comprised of a single filament that is smooth and easily passes through tissue. The disadvantage is that monofilaments tend to be stiff, and knots can slip. Polyfilament sutures are braided and usually easier to handle with better knot security; however, the interstices between the filaments can provide an area for bacterial growth and are not recommended for placement in skin.

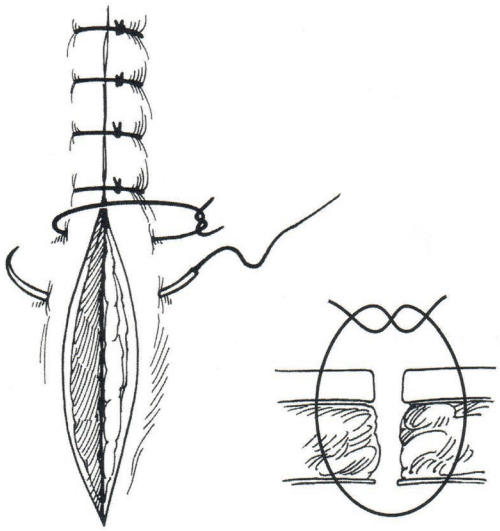
General characteristics of common sutures used in laboratory animal surgery:

	Name	Composition	Configuration	Tissue Reactivity	Handling	Knot Security	Comments
Absorbable	Vicryl WebCryl	Polyglactin 910	Braided	Low	++++	+++	50% loss of tensile strength in 3 weeks Absorbed in 56-70 days
	Monocryl MonoWeb	Poliglecaprone 25	Monofilament	Low	++	++	50% loss of tensile strength in 2 weeks Absorbed in 119 days
	PDS II WebMax	Polydioxanone	Monofilament	Low	+++	++	50% loss of tensile strength in 5-6 weeks Absorbed in 180 days
Nonabsorbable	Silk	Silk	Braided	High	++++	+++	Causes foreign body Reaction with inflammation
	Ethilon WebLon	Nylon	Monofilament	Low	++	+	
	Prolene WebFil	Polypropylene	Monofilament	Low	+++	++	Very strong

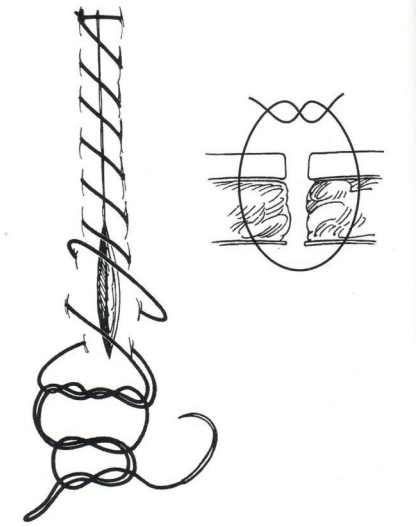
- Suture patterns: Most species will have incisions closed with either simple continuous or simple interrupted suture patterns. The ideal would closure involves maintaining tensile strength throughout the healing process and should be technically simple and quick for the surgeon to perform correctly allowing for precise skin margin approximation.

- Interrupted suture patterns: every suture has a knot allowing more precise wound margin apposition and improved incision closure security. Interrupted patterns generally take longer and are advocated for the skin, where dehiscence is a concern or areas of high tension. The most common interrupted pattern is the simple interrupted, but other patterns include mattress patterns, cruciate and Lembert.
- Continuous suture patterns are generally reserved for closure of non-cutaneous such as intradermal sites, subcutaneous tissue and body wall. Continuous patterns are generally faster to insert and distribute tension along the entire length of the surgical wound. Care must be taken to avoid damage to the suture material during placement of continuous sutures. The most common continuous pattern is the simple continuous, other patterns include Cushing, Connell, continuous locking and mattress pattern.

Figures show simple interrupted and simple continuous suture patterns:



Simple interrupted suture pattern with cross section of suture bite. Borrowed from Hendrickson: Techniques of Large animal Surgery, ed 3).



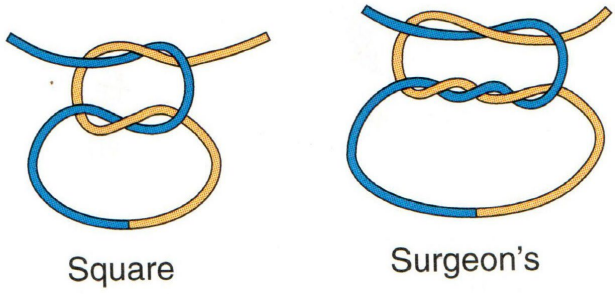
Simple continuous suture pattern with cross section of suture bite. Borrowed from Hendrickson: Techniques of Large animal Surgery, ed 3).

- Surgical Knots: the purpose of a surgical knot is to bind to suture ends. Surgically acceptable knots are square and surgeon's knots (see image below).
    - Square knot: a surgical square knot requires the formation of two opposing simple knots (throws). It is produced by reversing direction on each successive simple knot (throw) while maintaining even tension.

- Surgeon's knot: created by using a double loop as the first throw followed by simple knots as described above. Surgeon's knots improve knot security at the expense of greater knot and suture bulk. Surgeon's knots should only be used in areas where knot security is a concern such as high tension closures.

- Number of throws: the number of throws (simple knots) for a secure surgical knot can be dependent on the suture material and the wound characteristics (tension, location, etc). In general four to six throws are appropriate for surgical knots. More throws can be placed if there is a concern about knot security.

- Knot placement: surgical knots may be created by instrument or hand-tie methods. For the concern of potential injury from suture needles, instrument ties are advocated.



Square and Surgeon's knot. Borrowed from: Tobais and Johnston, Veterinary Surgery Small Animal, ed 1, 2012.

Some general recommendations are provided by species:

Species	Deeper Tissue/Abdominal Closure	Skin Closure
Mouse	• 6-0 to 4-0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25)_ • Simple interrupted or simple continuous	• 5-0 to 4-0 nylon or wound clips • Simple interrupted
Rat	• 5-0 to 3-0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25) • Simple interrupted or simple continuous	• 4-0 to 3-0 nylon or wound clips • Simple interrupted
Rabbit	• 4-0 to 2-0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25) or PDS II (polydioxanone) • Simple interrupted or simple continuous +/- intradermal	• 4-0 to 2-0 nylon • 4-0 stainless steel suture • Simple interrupted

Pig	• 3-0 to 0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25) or PDS II (polydioxanone) • Simple interrupted or simple continuous +/- intradermal	• 3-0 to 2-0 nylon • Simple interrupted or cruciate • Surgical staples
Marmoset	• 5-0 to 3-0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25) or PDS II (polydioxanone) • Simple interrupted or simple continuous +/- intradermal	• 4-0 to 3-0 nylon • Simple interrupted or cruciate
Macaque	• 3-0 to 0 Vicryl (polyglactin 910) or Monocryl (poliglecaprone 25) or PDS II (polydioxanone) • Simple interrupted or simple continuous +/- intradermal	• 3-0 to 0 nylon • Simple interrupted or cruciate • Surgical staples

- Tissue Adhesives (cyanoacrylate glue): Correct application of tissue glues is critical. Glue should be applied to incisions that have been closed with sutures that have no or low tension. The tissue glue should not be allowed to enter the wound but should form a bridge or barrier over the incision. If the glue enters in wound it may cause a chronic foreign body reaction with inflammation and delay healing. Application of tissue glue to a well apposed incision is advocated as it increases the speed of wound closure and creates a barrier to microbial penetration. Tissue glue should not be used if there is a concern for potential contamination of the surgical wound, as a replacement or supplementation for sutures in high tension areas or if there is concern for potential

Chapter 9: Guidelines for Analgesia, Anesthesia, Surgery and Post-operative Care in Rodents

Analgesic Protocols for Mice and Rats

Appropriate pain management is crucial for optimal postoperative recovery. Analgesics should minimally be provided for the time period outlined in the CAC protocol and then as needed. Make sure analgesics are given on time; recheck intervals should match when the next analgesic dose is due or at a minimum of every 24 hours after analgesic dosing stops. Recommendations by procedure are made in the following table. In addition to following these guidelines, animals should always be monitored after the procedure for signs of pain.

Analgesics **must be provided at the duration and dose as described in the CAC protocol.** Make sure analgesics are given on time, and ensure observations are performed at appropriate intervals and/or times of the day. For example, checking an animal twice per day would generally fall between 9-10 am then 3-4 pm. Ideally, the intervals that animals are observed are during their light cycle, and an assessment of behavior, the incision, and general appearance should be made.

Should animals be found to be painful after the initial analgesic is provided, please contact the DCM Veterinary Technicians to discuss options and/or obtain the approval for additional pain medication administration by the veterinarian. Should animals consistently have break-through pain or pain of a longer duration than expected, the CAC protocol should be amended with input from the overseeing veterinarian.

Rodent Procedure	**Expected Post-procedure Pain Level**	**Analgesic Options**
• IP, SQ, IM injection • Ear punch biopsy	No Pain to Minimal Pain	• Analgesia not required
• Intravenous blood collection and injections • Ear bar placement in stereotaxic frame	No Pain to Mild Pain	• Anesthesia may be required, see discussion on blood collection sites in mice and rats • In addition to general anesthesia, topical anesthesia is recommended for: 1. Retroorbital bleed or injection: 1 drop of proparacaine in eye 30 seconds before injection/bleed 2. Ear bar placement: Application of topical local anesthetic at least 10-15 minutes prior to placement of ear bars (Lidocaine 2.5%/Prilocaine 2.5% cream)
• Intraarticular (joint) injection • Subcutaneous tumor or device implant by large gauge needle • Tail tip amputation	Minimal Pain	• Analgesia not required, but anesthesia is required during procedure (not required for tailing in mice <14 days old)

• Tattoo		
• Skin incision only (< 2 cm) • Subcutaneous implant (pump, telemetry, pellet) • Punch biopsy of skin • Intra-ocular injection • Superficial tumor implant (single mammary fat pad injection)	Mild Pain (analgesia needs to last 8 hours)	**Option 1:** Local Anesthetic Bupivacaine 3 mg/kg or Lidocaine 4 mg/kg as local infiltration prior to incision
		Option 2: Single dose buprenorphine-HCl Mice: 0.05-0.1 mg/kg SQ or IP before procedure Rats: 0.01-0.05 mg/kg SQ or IP before procedure
		Option 3: Single dose NSAID (meloxicam or carprofen) before procedure • Meloxicam injectable: 2 mg/kg SQ (rat), 5 mg/kg SQ (mouse) OR • Carprofen injectable: 5 mg/kg SQ OR Meloxicam oral formulation: 2 mg/kg PO (rats) started morning before surgery
• Vascular catheter placement via cut-down • Large skin incision (>2 cm) • Castration/Vasectomy • Minor Laparotomy (ovariectomy, splenectomy or minor implant) • Minor craniotomy (virus injection, no implant) • Tissue dissection (multiple tumor induction sites or tumor removal) • Minor orthopedic procedure (soft tissue or cartilage only) • Skin grafts	Moderate Pain (analgesia needs to last 24-48 hours)	**Option 1:** Local anesthetic at the time of surgery and NSAID (carprofen or meloxicam) for 48 hours • Bupivacaine 3 mg/kg as local infiltration prior to incision AND • Meloxicam injectable: 2 mg/kg SQ (rat), 5 mg/kg SQ (mouse) OR Carprofen injectable: 5 mg/kg SQ every 24 hr OR • Meloxicam oral formulation: 2 mg/kg PO (rats) started morning before surgery
		Option 2: Buprenorphine-HCl for 24 hrs and NSAID (carprofen or meloxicam) for 48 hours • Buprenorphine HCl for 24 hrs Mice: 0.05-0.1 mg/kg SQ or IP before procedure and q 4-6 hrs for 24 hours Rats: 0.01-0.05 mg/kg SQ or IP before procedure and q 8-12 hrs for 24 hours AND • Meloxicam injectable: 2 mg/kg SQ (rat), 5 mg/kg SQ (mouse) OR Carprofen injectable: 5 mg/kg SQ every 24 hr OR Meloxicam oral formulation: 2 mg/kg PO (rats) started morning before surgery

		Option 3: Ethiqa XR Mice: 3.25 mg/kg SQ before procedure Rats: 0.65 mg/kg SQ before procedure • If needed, a single repeat dose of Ethiqa XR may be given after 72 hours. OR Buprenorphine-Sustained Release (SR) Mice: 0.5-1.0 mg/kg SQ before procedure Rats: 1.0-1.2 mg/kg SQ before procedure
• Thoracotomy • Major Laparotomy (C- • section, electroporation, large • visceral resections) • Major craniotomy (bone • removal +/- implant) • Laminectomy/Vertebral • Surgery • Major orthopedic (fracture, • amputation) • Major eye procedure • (enucleation, intraocular • implants)	Severe Pain (analgesia needs to last at 48-72 hrs)	**Option 1: Ethiqa XR or** Buprenorphine-SR and NSAID (carprofen or meloxicam) for at least 48 hours • Ethiqa XR (lasts 72 hours) Mice: 3.25 mg/kg SQ before procedure Rats: 0.65 mg/kg SQ before procedure • If needed, a single repeat dose of Ethiqa XR may be given after 72 hours. OR • Buprenorphine-SR (lasts 36-48 hours) Note: animals may need to be re-dosed after 36 hours Mice: 0.5-1.0 mg/kg SQ before procedure Rats: 1.0-1.2 mg/kg SQ before procedure AND • Meloxicam injectable: 2 mg/kg SQ (rat), 5 mg/kg SQ (mouse) OR • Carprofen injectable: 5 mg/kg SQ every 24 hr OR • Carprofen MediGel: one cup of MediGel CPF on the cage floor with the animals 24 hours prior to surgery and then another cup after surgery OR Meloxicam oral formulation: 2 mg/kg PO (rats) started morning before surgery
		Option 2 (if NSAIDs are contraindicated): Ethiqa XR or Buprenorphine-SR and local anesthetic at the time of surgery • Bupivacaine 3 mg/kg as local infiltration prior to incision or Liposomal bupivicaine (Exparel, Pacira) 1 mg/kg SQ AND • Ethiqa XR (lasts 72 hours) Mice: 3.25 mg/kg SQ before procedure Rats: 0.65 mg/kg SQ before procedure • If needed, a single repeat dose of Ethiqa XR may be given after 72 hours OR • Buprenorphine-SR (lasts 36-48 hours) Note: animals may need to be re-dosed after 36 hours Mice: 0.5-1.0 mg/kg SQ before procedure Rats: 1.0-1.2 mg/kg SQ before procedure

		Option 3: Ethiqa XR or Buprenorphine-SR and local anesthetic at the time of surgery followed by NSAID (meloxicam or carprofen) for at least 48 hours (see option 1 and 2 for dosing)

Note: Minor procedures result in minimal inflammation, rapid tissue healing and no chronic impairment. Major procedures require more extensive tissue resection and organ involvement that will likely result in prolonged inflammation and delayed healing.

Note 2: Additional guidance on timing of administration of medications when providing multimodal anesthesia (ie combinations of Ethiqa and meloxicam) may be provided by the DCM Pharmacy.

ROUTES OF ADMINISTRATION- "SQ" means subcutaneously, "IP" into the peritoneal cavity, "PO" by mouth, "IM" into a muscle, "IV" into a vein.

DOSING INTERVALS- "SID" means once a day (every 24 hours), "BID" twice a day (every 12 hours), "TID" three times a day (every 8 hours), "QID" four times a day (every 6 hours), "PRN" as needed; "Q X hr" means give every "X" hours.

Recommended Analgesics for Rodent Surgical Procedures:

The doses provided in the table above, as well as the recommendations below, are based on published texts assessing rodent analgesia; however, the dose needed for analgesia can be affected by strain, age and physiologic status. The DCM veterinary staff should be consulted if there are concerns regarding the dosing of analgesics.

Opioids

Buprenorphine is a partial μ agonist commonly used to reduce pain in laboratory rodents and is available in two formulations: Buprenorphine-HCl (Immediate Release) and Buprenorphine-SR (Sustained Release). These are controlled substances (by the DEA) that require usage logs and locked storage.

- ***Buprenorphine-HCl (Regular, or immediate release)***: Recent rodent studies indicates that therapeutic concentrations of regular buprenorphine are not maintained beyond 4-6 h. Because of this (frequent dosing requires frequent animal handling), it is NOT recommended for long-term analgesia in adult rodents. Buprenorphine-HCl comes as a 0.3 mg/ml solution and should be diluted for more accurate dosing. Diluted solutions must be stored in sterile glass vials and expire after 1 month, or at the expiration date of any components, whichever is sooner.
    - Dosages and Injection Volumes:
        - Mice: 0.05- 0.1 mg/kg given SQ or IP every 4-6 hours (recommended dose = 0.1 mg/kg). Recommended dilution is 1 part buprenorphine-HCl, 9 parts in sterile saline (final concentration = 0.03 mg/ml).

Mouse Weight (grams)	10-20	21-30	31-40	41-50	51-60
Bup-HCl injection volume (ml) [0.03 mg/ml solution] for **0.05 mg/kg dose**	0.03 ml	0.05 ml	0.06 ml	0.08 ml	0.1 ml

Bup-HCl injection volume (ml) [0.03 mg/ml solution] for **0.1 mg/kg dose**	0.06 ml	0.1 ml	0.13 ml	0.16 ml	0.2 ml

- Rats: 0.01-0.05 mg/kg given SQ or IP every 8-12 hours (recommended dose = 0.05 mg/kg). Recommended dilution is 1 part buprenorphine-HCl, 9 parts in sterile saline (final concentration = 0.03 mg/ml). Note: Bup-HCl may cause pica (eating of non-food substances) in rats; use lower dose for Sprague Dawley rats (0.02 mg/kg).

Rat Weight (grams)	100-150	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Bup-HCl volume (ml) [0.03 mg/ml] for **0.01 mg/kg dose**	0.05	0.06	0.08	0.1	0.11	0.13	0.15	0.16	0.18	0.2
Bup-HCl volume (ml) [0.03 mg/ml] for **0.05 mg/kg dose**	0.25	0.33	0.41	0.5	0.58	0.66	0.75	0.83	0.91	1

- Neonates (mice or rats): The use of opioids in pre-weanling rodents may affect their ability to nurse and therefore should be used with care. Consult a DCM veterinarian about using analgesics in neonates. Also the volumes for administration are very small, and must be diluted to achieve a reasonable working volume. Buprenorphine HCl should be administered SQ TID or QID.

- ***Ethiqa XR***: a pharmaceutical-grade, FDA-indexed extended-release formulation of buprenorphine approved for pain control in rats and mice from Fidelis Animal Health. Pharmaceutical-grade drugs should be used whenever possible unless there is acceptable scientific justification for the use of non-pharmaceutical grade that is approved by the CAC. Please refer to the CAC Policy on "Non-Pharmaceutical Grade Compound and Extra-Label Drug Use" for further information

  Please note that the dosages and dose volumes for Ethiqa XR and Bup-SR (now labeled Bup-ER-LAB) are different and not interchangeable. See dosages and injection volume guidelines below. This extended-release formulation reduces the amount of animal handling and has been shown to retain therapeutic concentrations for as long as 72 hours after the initial dose in mice and rats. If needed, a single repeat dose may be administered 72 hours after the initial dose under direct veterinary guidance.

  Ethiqa XR comes in a single concentration of 1.3 mg/ml. It must be given as a subcutaneous injection and **CANNOT be diluted**. The safety of Ethiqa XR has not been evaluated in pregnant, lactating, neonatal, or immune-compromised mice or rats.

  - Dosages and Injection Volumes:
    - Mice (*Mus* species): 3.25 mg/kg SQ

Mouse weight (grams)	Ethiqa XR 1.3 mg/ml Concentration
	SQ Injection Volume for 3.25 mg/kg dose
18-21 g	0.05 ml
22-25 g	0.06 ml
26-29 g	0.07 ml
30-33 g	0.08 ml
34-37 g	0.09 ml
38-41 g	0.10 ml
42-45 g	0.11 ml
46-49 g	0.12 ml
50-53 g	0.13 ml
54-57 g	0.14 ml

- Rats (*Rattus* species): 0.65 mg/kg SQ

Rat weight (grams)	Ethiqa XR 1.3 mg/ml Concentration
	SQ Injection Volume for 0.65 mg/kg dose
190-209 g	0.10 ml
210-229 g	0.11 ml
230-249 g	0.12 ml
250-269 g	0.13 ml
270-289 g	0.14 ml
290-309 g	0.15 ml
310-329 g	0.16 ml
330-349 g	0.17 ml
350-369 g	0.18 ml
370-389 g	0.19 ml
390-409 g	0.20 ml
410-429 g	0.21 ml
430-449 g	0.22 ml
450-469 g	0.23 ml
470-489 g	0.24 ml
490-509 g	0.25 ml
510-529 g	0.26 ml
530-549 g	0.27 ml
550-569 g	0.28 ml

- ***Buprenorphine-ER-Lab (Extended Release):*** Sustained-release formulations of buprenorphine (Bup-SR), now labeled Buprenorphine-ER-LAB (Bup-ER-LAB), reduce the amount of animal handling and have been shown to retain therapeutic concentrations

for as long as 48 hours. Bup-SR comes in two concentrations: 0.5 mg/ml and 1 mg/ml. It must be given as a subcutaneous injection and **CANNOT be diluted**. Reference "Buprenorphine SR Information Sheet" from ZooPharm labs prior to use for injection guidelines. (https://wildpharm.com/medications/labanimals/item/68-buprenorphine-sr-lab-5ml- 05mgml.html)

Because Bup-SR is a non-pharmaceutical grade drug, acceptable scientific justification is required for CAC approval for its use. Please refer to the CAC Policy on "Non-Pharmaceutical Grade Compound and Extra-Label Drug Use" for further information.

Buprenorphine-SR cannot be diluted and is therefore not recommended for neonates.

- Dosages and Injection Volumes:
  - Mice: 0.5-1 mg/kg SQ every 36-48 hours (recommended dose = 1 mg/kg), for more accurate dosing use 0.5 mg/ml concentration

Mouse Weight (grams)	**Bup-SR 0.5 mg/ml Concentration**		**Bup-SR 1 mg/ml Concentration**	
	SQ Injection volume (ml) for 0.5 mg/kg dose	SQ Injection volume (ml) for 1 mg/kg dose	SQ Injection volume (ml) for 0.5 mg/kg dose	SQ Injection volume (ml) for 1 mg/kg dose
10-20 g	0.02 ml	0.03- 0.04 ml	0.01 ml	0.02 ml
21-30 g	0.03 ml	0.05-0.06 ml	0.015 ml	0.03 ml
31-40 g	0.04 ml	0.07-0.08 ml	0.02 ml	0.04 ml
41-50 g	0.05 ml	0.09-0.1 ml	0.025 ml	0.05 ml
51-60 g	0.06 ml	0.11-0.12 ml	0.03 ml	0.06 ml

  - Rats: 1-1.2 mg/kg SQ every 48-72 hours, use 0.5 or 1 mg/ml concentration

Rat Weight (grams)	**Bup-SR 0.5 mg/ml Concentration**	**Bup-SR 1 mg/ml Concentration**
	SQ Injection volume (ml) for 1 mg/kg dose	SQ Injection volume (ml) for 1 mg/kg dose
100-150 g	0.3 ml	0.15 ml
151-200 g	0.4 ml	0.2 ml
201-250 g	0.5ml	0.25 ml
251-300 g	0.6 ml	0.3 ml
301-350 g	0.7 ml	0.35 ml
351-400 g	0.8 ml	0.4 ml
401-450 g	0.9 ml	0.45 ml
451-500 g	1 ml	0.5 ml
501-550 g	1.1 ml	0.55 ml
551-600 g	1.2 ml	0.6 ml

Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs have anti-inflammatory, analgesic, and antipyretic properties both at peripheral and central sites. Side effects such as increased bleeding time, gastrointestinal ulceration, and renal papillary necrosis can ccur at high doses, with prolonged use, and in animals that are dehydrated or have underlying disease. Care must be exercised when giving NSAIDs during periods of low blood pressure (e.g., anesthesia) or dehydration; fluids should be given concurrently.

Most drugs are available in oral or injectable formulations, and the duration of effect is usually 12-24 hours. Meloxicam and carprofen are two recommended NSAIDs for use in rodents.

- ***Meloxicam Injectable***: Supplied as a 5 mg/ml injectable solution. Recommended dilution is 1 part meloxicam, 4 parts in sterile saline (final concentration 1 mg/ml) mixed well prior to administration. Administer injectable solution subcutaneously in the scruff (loose skin on dorsal neck) using a 25 or 27g needle. Dosing recommendations for rats and mice differ, consult the tables for each species.
    - Dosage and Injection Volumes:
        - Mice: 5 mg/kg SQ every 24 hours, for more accurate dosing use 1 mg/ml (diluted) concentration

Mouse Weight (grams)	10-20 gr	21-30 gr	31-40 gr	41-50 gr	51-60 gr
Meloxicam SQ Injection Volume (ml) **[1 mg/ml solution]**	0.1	0.15	0.2	0.25	0.3

        - Rats: 2 mg/kg SQ every 24 hours, for more accurate dosing use 1 mg/ml (diluted) concentration

Rat Weight (grams)	100-150 gr	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Meloxicam SQ injection volume (ml) **[1 mg/ml]**	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2

    - ***Carprofen Injectable***: Supplied as a 50 mg/ml solution that must be kept refrigerated (4C). The drug is viscous and must be diluted. Recommended dilution is 1 part carprofen, 9 parts in sterile saline (final concentration 5 mg/ml) mixed well prior to administration. Administer injectable solution subcutaneously in the scruff (loose skin on dorsal neck) using a 25 or 27g needle.
        - Dosage and Injection Volumes:
            - Mice: 5 mg/kg SQ every 24 hours, for more accurate dosing use 5 mg/ml (diluted) concentration

Mouse Weight (grams)	10-20 gr	21-30 gr	31-40 gr	41-50 gr	51-60 gr
Carprofen SQ Injection Volume (ml) [5 mg/ml solution]	0.02	0.03	0.04	0.05	0.06

            - Rats: 5 mg/kg SQ every 24 hours, for more accurate dosing use 5 mg/ml (diluted) concentration

Rat Weight (grams)	100-150 gr	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Carprofen SQ injection volume (ml) [5 mg/ml]	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6

- Neonates (mice or rats): NSAID use has not been thoroughly investigated in neonates and may affect renal function and other systems dependent on prostaglandins for normal maturation. Consult a DCM veterinarian about using analgesics in neonates. The volumes for administration are very small, and should therefore be diluted to achieve a reasonable working volume.

- ***Meloxicam Oral Suspension for Rats***: 2 mg/kg PO once a day, for more accurate dosing use 1.5 mg/mL oral suspension. Larger volumes likely need to be split into multiple doses.

Rat Weight (grams)	100-150 gr	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Meloxicam PO dosing volume (ml) [1.5 mg/ml]	0.2	0.26	0.33	0.4	0.46	0.53	0.6	0.66	0.73	0.8

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Local Anesthetics – provide analgesia by acting on peripheral nerves to stop the transfer of pain to the central nervous system. There are several routes of administration and types of drugs. In rodents they are commonly applied topically (Lidocaine 2.5%/Prilocaine 2.5% cream) or via local infiltration at the surgery site. They should be utilized preemptively (before the surgical stimulus). Side effects are usually associated with high doses or intravenous administration and include hypotension, dysrhythmias, seizures, and CNS depression.

- ***Bupivacaine***: Supplied as a 0.5% (5 mg/ml) solution. Recommended dilution is 1 part bupivacaine, 2 parts in sterile water (final concentration 1.6 mg/ml) and injected at 3 mg/kg SQ space below the planned incision site. Time to onset of action is 5-10 minutes and duration of action is approximately 8 hours.
    - Dosage and Injection Volumes:
        - Mice: 3 mg/kg SQ at the surgical site injected after the first cycle of surgical scrub/alcohol rinse. For more accurate dosing use a diluted concentration of 1.6 mg/ml.

Mouse Weight (gr)	10-20 gr	21-30 gr	31-40 gr	41-50 gr	51-60 gr
Bupivacaine SQ Injection Volume (ml) at surgical site **[1.6 mg/ml solution]**	0.04	0.05	0.07	0.09	0.11

        - Rats: 3 mg/kg SQ at the surgical site injected after the first cycle of

surgical scrub/ alcohol rinse. Depending on the size of incision use 1.6 mg/ml (diluted) and 5 mg/ml (undiluted) for accurate dosing for incision coverage.

Rat Weight (grams)	100-150 gr	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Bupivacaine SQ volume (ml) **[1.6 mg/ml solution**]	0.28	0.375	0.47	0.56	0.65	0.75	0.84	0.93	1.0	1.1
Bupivacaine SQ volume (ml) **[5 mg/ml solution]**	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.3	0.33	0.36

- ***Lidocaine hydrochloride***: Supplied as a 1% (10 mg/ml) or 2% (20 mg/ml) solution. Recommended dilution for mice with 1% is 1 part Lidocaine, 5 parts in sterile water; or, for 2%, 1 part Lidocaine, 11.5 parts in sterile water (final concentration 1.6 mg/ml), recommended dilution for rats with 1% is 1 part Lidocaine, 1 part in sterile water; or, for 2%, 1 part Lidocaine, 3 parts in sterile water (final concentration 5 mg/ml) to be injected at 2-4 mg/kg SQ into the space below the planned incision site. Time to onset of action is 1-2 minutes and duration of action is approximately 0.5-2 hours.
    - Dosage and Injection Volumes:
        - Mice: 4 mg/kg SQ at the surgical site injected after the first cycle of surgical scrub/alcohol rinse. For more accurate dosing, use a 1.6 mg/ml dilution.

Mouse Weight (gr)	10-20 gr	21-30 gr	31-40 gr	41-50 gr	51-60 gr
Lidocaine SQ Injection Volume (ml) at surgical site **[1.6 mg/ml solution]**	0.04	0.06	0.09	0.11	0.14

        - Rats: 4 mg/kg SQ at the surgical site injected after the first cycle of surgical scrub/ alcohol rinse. Depending on the size of incision use 5 mg/ml (diluted) and 10 mg/ml (undiluted) for accurate dosing for incision coverage.

Rat Weight (grams)	100-150 gr	151-200 gr	201-250 gr	251-300 gr	301-350 gr	351-400 gr	401-450 gr	451-500 gr	501-550 gr	551-600 gr
Lidocaine SQ volume (ml) **[5 mg/ml solution]**	0.1	0.14	0.18	0.22	0.26	0.3	0.34	0.38	0.42	0.46
Lidocaine SQ volume (ml) **[10 mg/ml solution]**	0.05	0.07	0.09	0.11	0.13	0.15	0.18	0.19	0.21	0.23

- ***Proparacaine (0.5% Ophthalmic Solution)***: Apply 1 drop to the eye at a minimum of 30 seconds before retroorbital bleed or ocular procedure. Solution must be refrigerated.

- ***Lidocaine 2.5%/Prilocaine 2.5% cream***: Apply a small amount with swab to the outer ear canal prior to placing ear bars. This takes approximately 10-15 minutes for analgesic effects.

Anesthetic Protocols for Mice and Rats

Recommended Anesthetic Protocols

Many anesthetic protocols exist for mice and rats. Specialized anesthetic protocols can be developed in coordination with DCM veterinary staff and must be on an approved CAC protocol. Anesthetic agents include injectable and inhalant anesthetics. In general, inhalant anesthetics are advocated over injectable anesthetics. Inhalant anesthetics delivered through a controlled delivery system (vaporizer) allow for rapid induction, maintenance of anesthetic delivery throughout the procedure, supplementation of oxygen, ability to rapidly titrate anesthetic amount and rapid recovery from anesthesia. Injectable anesthetics offer the convenience of delivery and can be safe when correctly calculated by body weight and careful re-dosing when indicated.

Injectable anesthetics are not advocated for long procedures or procedures expected to cause severe pain due to the inability to easily titrate anesthetic depth during these procedures.

Hypothermia may be used as a means of anesthetizing neonatal mice and rats pups less than 6 days of age. Below are some recommended anesthetic protocols by species:

- Mice (>6 days of age):
    - Inhalant anesthesia (preferred): Isoflurane delivered in 100% oxygen by a controlled vaporizer. Induction at 3-5% with maintenance at 1.5-2% delivered via nose cone.
    - Injectable anesthetics:
        - Tribromoethanol (Avertin or TBE): Supplied as a nonpharmaceutical grade powder that must be made according to the protocol, 125-300 mg/kg IP, repeated dosing may cause death. Should only be used for short surgical procedures. Anesthetic duration is 15-45 minutes.
            - See protocol for use below.
        - Ketamine/Xylazine: Prepared as a diluted solution from stock ketamine and xylazine. Induction dose of ketamine 100 mg/kg and xylazine 10 mg/kg IP. Anesthetic duration is 30-40 minutes.
            - Repeated dosing is generally discouraged, but if needed ketamine alone should be given at 50 mg/kg IP.
            - Reversal: Xylazine can be reversed by alpha-2 antagonists. Reversal of xylazine is advocated at the end of surgical procedures allowing for rapid recovery. Reversal is accomplished by atipamezole 1 mg/kg IP.
- Rats:

- Inhalant anesthesia (preferred): Isoflurane delivered in 100% oxygen by a controlled vaporizer. Induction at 3-5% with maintenance at 1.5-2% delivered via nose cone.
- Injectable anesthetics:
  - Ketamine/Xylazine: Prepared as a diluted solution from stock ketamine and xylazine. Induction dose of ketamine 60-80 mg/kg and xylazine 5-10 mg/kg IP. Anesthetic duration is 20-30 minutes.
    - Repeated dosing is generally discouraged, but if needed ketamine alone should be given at 30-40 mg/kg IP.
    - Reversal: Xylazine can be reversed by alpha-2 antagonists. Reversal of xylazine is advocated at the end of surgical procedures allowing for rapid recovery. Reversal is accomplished by atipamezole 1 mg/kg IP or SC.
  - Pentobarbital: Due to the difficulty in obtaining pharmaceutical grade pentobarbital, it is currently not recommended for surgical procedures. If available, dosing is 30-40 mg/kg IP. Duration of anesthesia is 20-60 minutes.

Avertin Use in Mice

Tribromoethanol (Avertin) is a general anesthetic used in mice for surgical procedures lasting between 20-30 minutes. It reduces blood pressure and respiratory rate with minimal cardiopressive effects, and provides rapid induction and recovery with loss of reflex activity and good muscle relaxation. High or repeat doses have caused peritonitis, intestinal ileus and hepatic and renal damage; unpredictable results can occur in mice less than 16 days of age.

Tribromoethanol solution decomposes to the toxic metabolites hydrobromic acid and dibromoacetic acid in response to light and heat (>40° C). The presence of these metabolites should be assumed if the pH of the solution is less than 5. For these reasons, both concentrated and dilute (working) solutions should be kept refrigerated at 4° C, with vials wrapped in aluminum foil. The working solution's pH should be checked prior to use; dilute solutions decompose more quickly than concentrated solutions.

Solutions should be filtered through a 0.2um syringe filter prior to use.

A working solution of 1.25-2.0% can be used. The use of more dilute solutions is recommended because of the risk of increased mortality at higher concentrations. DCM staff has used a 2.0% working solution without any evidence of an increase in mortality. The instructions below are for a 1.25% and 2.0% working solution.

Materials needed

a. Avertin (2, 2, 2-Tribromoethanol) (Sigma Cat. # T48402)
b. Tert-amyl alcohol (Sigma Cat. # 240486)
c. Sterile water or isotonic saline

To make a stock/concentrated solution (1.6 g/ml)

Add 0.63 ml of tert-amyl alcohol using a glass pipette to 1 g of Avertin (or for larger volumes 15.5 ml and 25 g). Mix solution in a dark glass sterile bottle and stir until dissolved. This may take some time and can be left overnight. The solution is light sensitive and hydroscopic (takes up water) so keep it in a tightly sealed, dark bottle and limit exposure to air; at 4 °C, it can be kept for about a year. After refrigeration, the mixture will need to be dissolved again by mixing as it tends to crystallize. Warming the solution under water will help get it back into solution. Once a solution turns yellow, oxidization has occurred and it should be discarded.

From the stock solution, a working solution is made with a resulting concentration between 1.25– 2.0%. Examples of 1.25% and 2.0% working solutions are described below, along with the volumes needed for dosing based on the weight of a mouse.

To make a working / dilute solution of 1.25% (12.5 mg/ml)

Mix 0.04 ml of the stock solution with 5 ml of sterile normal saline or sterile water, pass through a 0.2 µ filter into a polypropylene tube (solution can degrade some plastics) or sterile glass vial and mix well. Keep covered in tinfoil. It is recommended to make the solution fresh daily or use within 1 week.

Dosing (500 mg/kg) IP:

It usually takes about 5 minutes for the mouse to be fully anesthetized, i.e. lack of toe pinch reflex. An additional 0.2-0.3 ml can be given as needed. Anesthesia usually lasts for about 20-30 minutes. There will be some strain variability so doses will need to be adjusted accordingly. The following dose chart works well for outbred mice. **Note that there is a wide dose range (125- 500 mg/kg) and responses vary depending on the strain/stock of mice**

Volumes to administer using a 1.25% solution:

Weight of mouse (g)	Dose (mL)		Weight of mouse (g)	Dose (mL)
20	0.80		36	1.44
21	0.84		37	1.48
22	0.88		38	1.52
23	0.92		39	1.56
24	0.96		40	1.60
25	1.00		41	1.64
26	1.04		42	1.68
27	1.08		43	1.72
28	1.12		44	1.76
29	1.16		45	1.80
30	1.20		46	1.84
31	1.24		47	1.88

32	1.28		48	1.92
33	1.32		49	1.96
34	1.36		50	2.00
35	1.40		51	2.04

To make a working /dilute solution of 2% (20 mg/ml)

Mix 0.06 ml of the stock solution with 5 ml of sterile normal saline or sterile water, pass through a 0.2 um filter into a polypropylene tube (solution can degrade some plastics) or sterile glass vial and mix well. Keep covered in tinfoil. It is recommended to either make solution fresh daily or use within 1 week.

Dosing (500 mg/kg) IP:

It usually takes about 5 minutes for the mouse to be fully anesthetized, i.e. lack of toe pinch reflex. An additional 0.2-0.3 ml can be given as needed. Anesthesia usually lasts for about 20-30 minutes. There will be some strain variability so doses will need to be adjusted accordingly. The following dose chart works well for outbred mice. **Note that there is a wide dose range (125-500 mg/kg) and responses vary depending on the strain/stock of mice.**

Volumes to administer using a 2% solution:

Weight of mouse (g)	Dose (mL)	Weight of mouse (g)	Dose (mL)
20	0.50	36	0.90
21	0.52	37	0.92
22	0.55	38	0.95
23	0.57	39	0.97
24	0.60	40	1.00
25	0.62	41	1.02
26	0.65	42	1.05
27	0.67	43	1.07
28	0.70	44	1.10
29	0.72	45	1.12
30	0.75	46	1.15
31	0.77	47	1.17
32	0.80	48	1.20
33	0.82	49	1.22
34	0.85	50	1.25
35	0.87	51	1.27

Hypothermia for Neonatal Rodent Anesthesia

Hypothermia, produced by chilling an animal to near freezing temperature, may be used as a means of anesthetizing neonatal mice and rats pups less than 6 days of age. Their small size and body mass makes rapid cooling feasible through surface cooling, and they are resistant to arrest of blood supply to the brain and tolerate extended periods of 1º C body temperature without known negative effects. **Potential risks of hypothermia include superficial tissue damage, ventricular fibrillation, tissue hypoxia and metabolic acidosis on warming.**

When a need for hypothermia is demonstrated and described in an approved CAC protocol, the following guidelines should be followed:

- To induce hypothermia, pups may be placed in a latex glove (cut off a finger for this) and immersed up to the neck in crushed ice and water (2º C to 3º C) which requires a 5-8 minute induction time (2-3 minutes to unconsciousness and 3-5 minutes to complete blockage of neural transmission). Alternatively, pups may be placed in a paper–lined tube and packed in crushed ice; this method may require up to 15 minutes for a surgical plane of anesthesia. Analgesia for hypothermia induced by these methods lasts approximately 10 minutes.
- Do not place the animals directly on the cooling medium; provide a cloth, paper, or other barrier material. Simply placing conscious animals in a cold room or on an ice pack are unacceptable as induction may take 30-45 minutes.
- The anesthetic state may be prolonged by placing the hypothermic pup on an ice pack (3º C to 4º C). Studies have shown that rodent pups will maintain a core body temperature of approximately 5º C when kept on an ice pack for a maximum of 15 minutes.
- Illumination of the surgical field should be fiber optic in nature because incandescent bulbs may cause inadvertent and uncontrollable warming.
- After the procedure, avoid rapid re-warming because of possible tissue damage. Until animals are fully conscious, they cannot escape excessive heat generated by incandescent lamps, heating pads, or other warming devices. Pups can also be placed in an incubator at 33º C for 20 to 30 minutes. Complete recovery typically requires 30 to 60 minutes.
- The recovering animals may benefit from supplemental oxygen.

Guidelines for Surgery in Mice and Rats

Successful surgical manipulations are dependent on assessment of animal health pre-operatively, maintenance of adequate and stable anesthesia, smooth recovery from anesthesia and post-operative care.

Pre-Anesthetic Planning

- Pre-anesthetic assessment and planning: For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. See acclimation times for each species before experimental manipulation. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere. Preoperative fasting is not necessary in these species and should only be done if justified and

approved in the animal protocol.

- Scheduling invasive surgical procedures (with likelihood of morbidity) on Fridays, weekends or holidays is strongly discouraged. Overlap of invasive procedures with holidays and weekends makes post-op monitoring by researchers and veterinary support more difficult. Postoperative animals must be checked on weekends by the investigator at intervals that match when analgesia is due, or at a minimum of every 24 hours.

- Preparation of the Surgical Work Area:
  - Prior to and after the completion of all surgical procedures, all organic debris should be removed and cleaned from work surfaces with a disinfectant (accelerated hydrogen peroxide, fresh 10% bleach, or similar) solution for the appropriate contact time. Covering the work surface with clean plastic-backed absorbent paper (diaper, blue pad) or an equivalent for each procedure is recommended. Any devices or equipment, e.g., animal restraining devices, monitoring equipment, stereotaxic devices, that will be required in the surgical field should be disinfected. The above practices are performed in order to reduce or eliminate potentially infectious organisms and their substrates.
  - Aseptic rodent surgery should be conducted in a dedicated area of the laboratory that is free of materials not directly involved in the surgical procedure, out of main traffic patterns in the room and not observable by the general public and constructed of non- porous, washable surfaces. An effort should be made to designate three spaces for a surgical procedure.
    - Animal preparation for shaving and aseptic preparation of the skin.
    - Surgical area where equipment may be needed, e.g., stereotaxic frame/microscope.
    - Postoperative recovery area.

Guidelines for Surgery in Gerbils and Hamsters

Successful surgical manipulations are dependent on assessment of animal health pre-operatively, maintenance of adequate and stable anesthesia, smooth recovery from anesthesia and post-operative care.

Pre-anesthetic Assessment and Planning:

For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. See acclimation times for each species before experimental manipulation. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere. Preoperative fasting is not necessary in these species and should only be done if justified and approved in the animal protocol.

1. Analgesia: Appropriate pain management is crucial for optimal postoperative recovery. Analgesics should minimally be provided for the time period outlined in the CAC protocol and then as needed. Make sure analgesics are given on time; recheck intervals should match when the next analgesic dose is due or at a minimum of every 24 hours after analgesic dosing stops. Recommendations by procedure are made in the following

table:

Mild Pain (analgesia needs to last 8- 24 hours)	Option 1: Local Anesthetic • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision
	Option 2: Buprenorphine-HCl • Buprenorphine HCl 0.01-0.05 mg/kg SQ or IP every 6-12 hours for 1-2 doses
	Option 3: Single dose NSAID before procedure • Meloxicam injectable: 2 mg/kg SQ (hamster and gerbil), 0.3 mg/kg SC (guinea pig) before procedure OR • Carprofen injectable: 5 mg/kg SQ before procedure
Moderate Pain (analgesia needs to last 24- 48 hours)	Option 1: Local anesthetic prior to incision and NSAID for 48 hours • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision AND • Meloxicam injectable: 2 mg/kg SQ (hamster and gerbil), 0.3 mg/kg SC (guinea pig) every 24 hours OR • Carprofen injectable: 5 mg/kg SQ every 24 hours
	Option 2: Buprenorphine-HCl for 24-48 hours and NSAID for 48 hours • Buprenorphine HCl 0.01-0.05 mg/kg SQ or IP every 6-12 hours AND • Meloxicam injectable: 2 mg/kg SQ (hamster and gerbil), 0.3 mg/kg SC (guinea pig) every 24 hours OR • Carprofen injectable: 5 mg/kg SC every 24 hours
Severe Pain (analgesia to last at least 48- 72 hours)	Option 1: Buprenorphine-HCl for 48-72 hours and NSAID for 48-72 hours • Buprenorphine HCl 0.01-0.05 mg/kg SQ or IP every 6-12 hours AND • Meloxicam injectable: 2 mg/kg SQ (hamster and gerbil), 0.3 mg/kg SQ (guinea pig) every 24 hours OR • Carprofen injectable: 5 mg/kg SQ every 24 hours
	Option 2: Local anesthetic prior to incision, Buprenorphine-HCl for 48-72 hours and NSAID for 48-72 hours • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision AND • Buprenorphine HCl 0.01-0.05 mg/kg SQ or IP every 6-12 hours AND • Meloxicam injectable: 2 mg/kg SQ (hamster and gerbil), 0.3 mg/kg SQ(guinea pig) every 24 hours OR • Carprofen injectable: 5 mg/kg SQ every 24 hours

2. Anesthesia: Many anesthetic protocols exist for mice, rats, hamsters and guinea pigs and specialized anesthetic protocols can be developed in coordination with DCM veterinary

staff and must be on an approved CAC protocol. Anesthetic agents include injectable and inhalant anesthetics. In general, inhalant anesthetics are advocated over injectable anesthetics. Inhalant anesthetics delivered through a controlled delivery system (vaporizer) allow for rapid induction, maintenance of anesthetic delivery throughout the procedure, supplementation of oxygen, ability to rapidly titrate anesthetic amount and rapid recovery from anesthesia. Injectable anesthetics offer the convenience of delivery and can be safe when correctly calculated by body weight and careful re-dosing when indicated. Injectable anesthetics are not advocated for long procedures or procedures expected to cause severe pain due to the inability to easily titrate anesthetic depth during these procedures. Below are some recommending anesthetic protocols by species:

a. Injectable anesthetic protocols may be developed with coordination of DCM veterinary staff. Inhalant anesthetics are preferred.

b. Inhalant anesthesia: Isoflurane delivered in 100% oxygen by a controlled vaporizer. Induction at 3-5% with maintenance at 1.5-2% delivered via nose cone or facemask.

3. Intraoperative support and monitoring: Monitoring of animals during anesthesia includes evaluation of respiratory rate and character, color of the skin and mucous membranes and lack of response to surgical stimulus. Pedal withdrawal reflex (pinnae reflex in guinea pigs) is evaluated at the start of procedure and when anesthetic depth is in question. Pulse oximetry is available and can be used to assess oxygen saturation, respiratory rate and pulse rate. **Thermal support is required for all surgical procedures as hypothermia will occur with all anesthetic protocols. Temperature controlled thermal pads are recommended.**

4. Recovery and post-operative support: Animals should be kept warm through the use of heating pads, blankets, or lamps, and, if animal size permits, body temperature should be monitored and recorded until it returns to normal. Be careful during preparation of the surgical site to not get rodents excessively wet, which leads to hypothermia. Also do not to overheat the animal, i.e., cautious use of heat lamps and electric blankets.

    a. Animals recovering from anesthesia should be rotated from side to side every half hour until they are able to maintain sternal recumbency. Frequency of observation is identical to that described for non-rodent mammalian species.

    b. Hydration should be assessed on a daily basis and fluid replacement administered at a volume of 60 ml/kg body weight per day for animals that are not eating and drinking postoperatively. In small laboratory animals, fluids may be given SQ or IP. Lactated Ringer's solution or equivalent should be used. Rodents having major survival surgery are likely to benefit from warm fluids given subcutaneously at the completion of surgery at a rate of 20 ml/kg body weight.

    c. Adequate nutrition is necessary in the healing animal patient. Caloric replacement should be instituted for animals that have not resumed eating by the second postoperative day; this may require supplemental feedings using specialized dietary formulations and feeding methods. Diet Gels are available in the animal facilities for additional support.

    d. Please contact the facility veterinary technician or place a health check card if you

have concerns about the post-operative recovery of the animals. Analgesic delivery in the post-operative period should match what is on the approved CAC protocol.

Surgical Preparation

1. Pre-surgical Preparation of the Animal:
    a. Ascertain that the animal is properly anesthetized before starting procedures. This can be done by determining the response to a painful stimulus (toe pinch, Heart rate, respiratory rate, and blood pressure are also useful parameters to determine depth of anesthesia.
    b. After anesthetic induction, administer eye lubrication, e.g., Puralube, to prevent the occurrence of corneal ulcers or other eye damage. The blink response is generally blunted and the eyes may remain open.
    c. Provide a heat source or heat sources before beginning the surgical preparation of the animal. Temperature controlled thermal support is recommended.
    d. Analgesia (pain relief) is generally required and should be administered prior to surgery, or at minimum, prior to recovery to allow effective systemic levels to be obtained before return of consciousness.
    e. Hydration: Rodents having major survival surgery are likely to benefit from warm fluids given subcutaneously at the beginning of surgery at a rate of 20 ml/kg body weight.
2. Preparation of the Surgical Site:
    a. Proper surgical preparation must be performed as outlined above with the removal of hair/feathers using either small clippers, depilatory cream in rodents or plucking feathers in birds. The size of the cleared area should be sufficient to ensure that the surgeon has adequate visibility of structures beneath the skin; the incision can be enlarged without compromising the sterile field if complications develop; and hair/feathers are not inadvertently incorporated in wound closure.
    b. The entire cleared area should be prepared with either povidone-iodine scrub (Betadine scrub) or chlorhexidine scrub (Nolvasan scrub). Using cotton-tip applicators or cotton balls, the area should be scrubbed beginning at the center of the incision site and working out to the perimeter or in a manner appropriate for the area to prevent the contamination of the site of the surgical incision. After a minimum of three cycles and contact time of three minutes with skin scrubs, a clean applicator or cotton ball should be selected soaked with 70% isopropyl alcohol or 70% ethanol (sterile water or saline should be used for rinsing mucous membranes or open wounds).
    c. Draping: The surgical site should be draped with sterile disposable, water-impermeable paper, or plastic drape sufficient to exclude surrounding hair/feathers from the incision and large enough to ensure a working sterile field around the subject. This is especially important when skin incisions are being closed. It is recommended to have a minimum of a 8” square drape for mice and 12” square drape for rats and birds.

3. Surgeon Preparation:
    a. Individuals participating in actual surgical manipulation must wear a clean lab coat, sterile gloves, a hair bonnet, and a face mask. Hands should be washed with soap prior to gloving. For rodent surgeries, 70% alcohol-based hand sanitizer may be used as an alternative to disinfectant soap. When performing multiple surgeries on rodents/birds in 1 session, new gloves should be used for each animal.
4. Intraoperative support and monitoring:
    a. Monitoring of animals during anesthesia includes evaluation of respiratory rate and character, color of the skin and mucous membranes and lack of response to surgical stimulus. The bilateral pedal withdrawal reflex is evaluated at the start of the procedure and when the depth of anesthesia is in question. Pulse oximetry is available and can be used to assess oxygen saturation, respiratory rate and pulse rate.
    b. Thermal support is required for all surgical procedures as hypothermia will occur with all anesthetic protocols. Temperature controlled thermal pads are recommended. See below for recommendations and indications.
5. Recovery:
    a. Animals should be kept warm through the use of recirculating warm water heating blankets or steady-state thermal warming pads (preferred). Incandescent bulbs, heat lamps and electric heat pads are discouraged from being used for warming animals. If animal size permits, body temperature should be monitored and recorded until it returns to normal. Be careful during preparation of the surgical site not to get rodents excessively wet, which can lead to hypothermia. Also do not overheat the animal, i.e., cautious use of heat lamps and electric blankets.
    b. Cages of recovering animals should be put half-on / half-off the warming device to allow animals to move to a cooler spot in the cage, voluntarily, should they become too warm.
    c. Animals recovering from anesthesia should be rotated from side to side every half hour until they are able to maintain sternal recumbency. Frequency of observation is identical to that described for non-rodent mammalian species.
    d. Sutures and wound clips must be sterile and be removed from skin when healing is complete, usually 7 to 14 days.
    e. Place and complete surgical postoperative cards. Place the unique surgery card number on the “Animal Health Monitoring” sheet on the room door. Postoperative monitoring for a minimum of three days is required in rodents with surgical procedures. Note activity, pain, hydration and incision condition. Analgesia dosing and other treatments must also be recorded on the post-op monitoring card, along with the time and the investigator's initials.
    f. Please contact the facility veterinary technician or place a health check card if you

have concerns about the post-operative recovery of the animals. Analgesic delivery in the post-operative period should align with the approved CAC protocol.

Post-Operative Care for Rodents

It is the responsibility of the investigator, in consultation with the clinical veterinarian and animal care staff, to promote the optimal recovery of the animal from surgery and anesthesia by providing appropriate postoperative care. A number of factors, e.g., type of surgery performed, type and amount of anesthetic used, will modify the nature, duration and intensity of the postoperative care required by the patient.

Postoperative care programs should be considered and designed before commencing any experimental procedures and must be described in a CAC-approved protocol.

Considerations of Hypothermia:

Mice have a large body surface-to-body weight ratio; as a result, they become hypothermic very quickly once anesthetized. This is particularly true in animals undergoing surgeries that involve opening a major body cavity. Heat can also be lost by evaporation and conduction from the ears, tail and feet. Considerations for preventing hypothermia are listed below.

1. Optimize surgical preparation, technique, and recovery: The recovery area should be quiet and away from loud noise. Use of excessive aseptic agents or excess fluid on fur surrounding the surgical site can increase the risk of hypothermia and should be avoided. In addition, the surgical scrub and alcohol can be pre-warmed before application to the mouse or rat. Make sure the animal is dry after surgery before placing into the recovery cage.
2. Provide supplemental heat: Supplemental heat is required for rodent surgeries. During surgery, heat loss can be minimized by minimizing organ exposure from body cavities and by administering warmed subcutaneous or intraperitoneal fluids intra- and/or postoperatively. Supplemental heat must also be provided intraoperatively and postoperatively at least until the animals recover. Depending on the procedure, choice of analgesics, and health of the animal, some rodents recover better when supplemental heat is maintained for prolonged periods (hours/days). Examples of supplemental heat sources are provided below. **Regardless of the heat source, cages should be placed in a way that allows part of the cage to remain unheated so that the animal can escape the heat if desired.**
3. Recirculating water blankets: DCM highly recommends using water blankets over the other options. Frequently used during and after surgery on the “low” heat setting. Check for leaks prior to use.
   Recommended model:

   Gaymar Heating/Cooling T/Pump
4. Hand warmers**:** Provide prolonged heat to a portion of the cage bottom when needed. **Place <u>underneath</u> a portion of the cage, NOT inside the cage.**

5. Heat lamps and electrical heating pads: Use of these items is discouraged.

Considerations of Hypoglycemia:

Decreased appetite and anorexia are common after surgery and may be due to pain and/or side effects of certain analgesics. Prolonged anorexia and hypoglycemia can lead to lethargy, poor body condition, and excessive weight loss requiring euthanasia. Considerations for preventing hypoglycemia are listed below.

1. Wet food pellets on the floor of the cage: Encourage eating and support animals during the post-operative period by providing an easily accessible food source from food hoppers, moistened with a water bottle or lixit placed within easy reach for mice or rats.
2. Diet supplements/gels: Recovery gels available from e.g. ClearH2O for 2-3 days postop may encourage eating. Consult with the veterinary staff to discuss options. Special diets should be started 1-2 days prior to surgery to acclimate the animals.

Considerations of Dehydration:

Dehydration can occur intraoperatively as a result of blood loss during surgery, fluid loss from evaporation when body cavities are opened, and postoperatively when animals are too depressed/lethargic to access their water bottles. Rodents having major survival surgery will benefit from warm fluids given subcutaneously at the beginning of surgery at a dose of 20 ml/kg body weight. Hydration should be assessed on a daily basis after surgery and fluid replacement administered. In small laboratory animals, fluids can be given subcutaneously or intraperitoneally. Sterile Lactated Ringer's solution or an equivalent should be used. Remember that multiple-use fluid bags must be maintained aseptically and require an expiration date of one month after initial penetration (see section on fluid therapy for more information). Hydrogel placed on the bottom of the cage provides easy access to fluids for animals that are likely to be painful after surgery and reluctant to drink from the water bottle.

Rodent-Specific Responses to Pain

When assessing pain in animals, it is assumed that procedures that cause pain in humans may cause pain in animals unless proven otherwise; however, it is also important to try and recognize species-specific variations. For instance, assessing pain in rodents and other prey species can be difficult because they minimize pain-associated behaviors unless the pain is incapacitating.

Rodents may show "normal" behavior as an inherent response to avoid predation.

Signs of potentially painful mice, gerbils, and hamsters are:

1. Poor nest building: animals will have intact nestlets or other bedding substrates
2. Decreased activity: reluctance to move around the cage during observation without disturbing the animal
3. Hunched posture
4. Ruffled or greasy hair coat

5. Abnormal gait, writhing, belly-pressing, flinching
6. Eyes partially closed, ears rotated back or down
7. Rapid, shallow breathing
8. Isolating themselves from cage mates
9. Reduced food/water intake – Note: opioids may reduce food and water intake if doses are excessive
10. Excessive licking or scratching; self-mutilation
11. Restlessness
12. Increased aggression or vocalization when handled

Below are signs of potentially painful rats:

- Decreased activity
- Hunched posture
- Porphyrin secretions at eyes/nares
- Abnormal gait, horizontal stretching, writhing, flinching, falling/staggering
- Eyes partially closed, ears back or down
- Piloerection, ruffled or greasy hair coat
- Rapid, shallow breathing
- Isolating themselves from cage mates
- Excessive licking or scratching; self-mutilation
- Increased aggression when handled
- Restlessness
- Reduced food/water intake – Note: Opioids may reduce food and water intake if doses are excessive

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Additional Considerations for Rodent Surgical Procedures

Intubation

Rodents are rarely intubated unless a thoracotomy is performed that would require the animal to be ventilated. When needed, intubation can be done using various different techniques and requires some practice. A modified otoscope blade or a small laryngoscope blade can be used. Transillumination of the larynx by an external light source can help with visualization. In addition, there is a commercial system that uses a fiber optic light that fits within the lumen of a cannula to assist with visualizing the glottis. With the animal in dorsal recumbency or suspended upright, the glottis can be visualized and a vascular catheter passed (16-18 gauge in rats, 22-24 gauge in mice). Care must be taken to avoid cannulating a bronchus. The cannula should be secured with tape to avoid inadvertent extubation.

Multiple Survival Surgeries

Multiple survival surgeries require scientific justification. See the CAC policy "Multiple Major, Minor, Multiple & Other Surgeries" for more information.

Non-Survival Surgeries

Non-recovery surgical procedures, which have been described in an approved CAC protocol, may be performed in a CAC-approved laboratory or in the dedicated surgical facilities within DCM. If performed in the DCM surgical facility, the procedures must be carried out using strict aseptic procedure. It is strongly recommended that all surgical procedures, even if terminal and taking place in a research laboratory, be performed as aseptically as possible to minimize experimental variables that might influence experimental results, e.g., terminal septicemia.

Surgery Cards

White surgery cards are placed by the researcher to alert DCM personnel that an animal has had surgery and requires special attention. Surgery cards are required for all rodent and non-mammalian surgeries. Postoperative notes and treatments are recorded on the card (front and back). After each monitoring session is completed, each surgery card must be signed and documented daily. Failure to sign them can result in a safeguard fee of $25 per day for each cage. The white surgery card will remain in front of the animal cage card until the animal has fully recovered and the sutures/wound clips are removed; then the surgery card can be placed in the

researcher’s notebook or behind the cage card.

Chapter 10: Guidelines for Analgesia, Anesthesia, Surgery and Post-operative Care in Species other than Rodents

Pre-Anesthetic Planning

- Pre-anesthetic assessment and planning: For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. See acclimation times for each species before experimental manipulation. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere.
    - ***DCM Policy for Elective Surgery on Fridays:*** Elective surgery of an invasive nature (i.e., a major operative procedure) and surgeries which may be of a less invasive nature are in general, not scheduled on Fridays in either of DCM's centralized operating rooms. Extenuating circumstances can be discussed with the veterinarian of the pertinent DCM surgery facility and will be evaluated on a case-by-case basis. Emergency procedures and, in general, rodent surgery are exempt from this policy. Invasive procedures on rodents will be prohibited, however, when morbidity resulting from such procedures is expected.

Fasting

Pre-anesthetic fasting in rabbits is generally not necessary because they have an anatomical feature called the limiting ridge between the esophagus and the stomach which prevents vomiting. If needed, rabbits may have food withheld for 12-24 hours prior to general anesthesia. This will not completely empty the stomach but will allow a more accurate anesthetic dose determination and reduce the impediment that abdominal viscera place on diaphragmatic excursion. Young rabbits should not have food withheld for more than 12 hours.

Monogastric animals, e.g., swine & nonhuman primates, must have food withheld for at least four hours prior to surgery (survival or non-survival), ideally 8-12 hours. This will help to prevent vomiting and the potential for aspiration during induction or recovery. All animals must have water available prior to the procedure unless special circumstances warrant its being withheld.

Pregnant animals of all species should not be fasted as this can lead to severe metabolic disturbances.

Premedication

Several classes of drugs are typically used before surgery in order to promote homeostasis during anesthesia, provide protection against infection, and reduce pain and/or anxiety. The use of these agents should be discussed with surgical veterinarians in the pre-planning stage and should be described in protocol submissions to the CAC. Veterinary staff can advise researchers on the appropriate use of these agents.

Parasympatholytic drugs can be given prior to general anesthesia in all animals except

ruminants. These drugs reduce respiratory secretions and eliminate vagal reflexes that may occur during intubation, ophthalmic pressure or visceral traction. Both drugs are given intramuscularly or subcutaneously. Rabbits may have circulating atropine-destroying substances (atropinases) and may require additional doses; glycopyrrolate can be used in the rabbit instead.

There is controversy surrounding **the use of antibiotics** in perioperative patients. Opponents argue that aseptic technique should obviate the need for antibiotics. If antibiotics are used, they should be present in the animal's body at an appropriate dose at the time of surgery. Infections that occur in spite of routine prophylaxis should be treated in accordance with the recommendations of the surgical veterinarian. All plans for pre-emptive antibiotic use must be described in the CAC protocol.

The use of **preoperative analgesics**, either alone or in combination with tranquilizers, reduces animal anxiety, decreases the total amount of anesthetic required, and provides post-operative pain relief in procedures of short duration. There is considerable interspecies variation in the dose, duration of action, and appropriateness of these drugs. Details of drug choice, timing of administration and dosage must be described to the CAC. Analgesics should routinely be administered pre- or intraoperatively for survival surgeries unless scientific justification is provided to the CAC that it would interfere with experimental objectives. **Continued dosing postoperatively should be guided by evaluating each animal for evidence of pain; if in doubt, analgesics should be administered.**

Endotracheal Intubation

The placement of an endotracheal tube helps insure a patent airway. **The following animals should be intubated when being manipulated in major surgical procedures: rabbits, marmosets, swine, and macaques.** Animals that are administered inhalant anesthetics are usually intubated depending on species and procedure. Endotracheal tubes should be of the cuffed variety if tracheal size permits. Prior to insertion, endotracheal tubes may be sparingly lubricated depending on species, preferably with lidocaine gel if endotracheal tube size permits. Cetacaine spray, a topical anesthetic agent, should only be used in those species susceptible to laryngospasm, e.g., macaques and swine. Inflation of the cuff occludes the space between the tracheal mucosa and the outer wall of the tube. Failure to inflate the cuff permits aspiration of pharyngeal secretions, allows anesthetic gases to be diluted by room air during inspiration, and contributes to operating theater pollution by anesthetic gases upon expiration. Excessive cuff inflation should also be avoided.

Tubes should only be advanced to the level of the thoracic inlet. This will avoid the inadvertent intubation of a mainstem bronchus. During stereotaxic procedures, wire-reinforced endotracheal tubes eliminate the possibility of kinking with subsequent airway obstruction.

In the rabbit, endotracheal intubation can be facilitated by using a Wisconsin #1 laryngoscope blade. A blunt stylet can be used as a guide for the endotracheal tube. Blind intubation techniques or use of a laryngeal airway mask may also be used successfully.

Endotracheal tubes should be evaluated for patency during the surgical procedure when possible. Suction catheters may be required to clear accumulated debris from the lumen of the

endotracheal tube. Additional atropine or glycopyrrolate, given intraoperatively, is useful in controlling respiratory secretions.

Endotracheal tubes are removed only when an animal has regained the swallowing reflex, and the cuff must be deflated prior to tube removal.

Fluid Therapy

All non-rodent mammals that undergo major surgical procedures should receive intravenous fluids delivered through catheters unless otherwise described and approved in the protocol. Common sites for catheterization include the cephalic, saphenous, femoral and jugular veins. Ear veins (rabbit, pig) and dorsolateral tail veins (ferret) can also be used. The provision of vascular access allows the anesthetist to maintain normovolemia and to administer drugs. Polyionic replacement fluids like Lactated Ringer's solution are preferred for intraoperative use unless specific disease states make other formulations more desirable. An initial rate of 3-5 ml/kg/hr is recommended for procedures involving a laparotomy and/or thoracotomy. Fluid administration may need to be tapered as hemodynamic parameters change in long procedures. Blood loss should be estimated during surgery, as animals should receive three times their estimated blood loss during the operative procedure. Fluids should also be administered during neurosurgical procedures; an infusion rate of 2-3 ml/kg/hr will not result in cerebral edema. If available, a fluid line warmer should be used to warm the fluids prior to intravenous delivery.

Operating Room Procedures

Only investigators with appropriate training will be allowed to perform surgery. Activities shall be directed by the surgeon and every effort should be made to minimize surgery time and tissue trauma. Closure techniques must be in accordance with accepted surgical practice. Procedures must be conducted using aseptic technique, which is designed to reduce post-surgical complications (e.g., infections, wound dehiscence), improve animal survival rates, and hasten animal return to basal physiological functions present prior to surgery.

Preparation of the Surgical Area:

Prior to and after the completion of all surgical procedures, all organic debris should be removed and cleaned from work surfaces with accelerated hydrogen peroxide (Peroxigard) or similar disinfectant solution. Any devices or equipment, e.g., animal restraining devices, monitoring equipment, stereotaxic devices, that will be required in the surgical field must be disinfected. The above practices are performed in order to reduce or eliminate potentially infectious organisms and their substrates.

Specialized surgical facilities are required for rabbits and other non-rodent mammalian species. All procedures must be conducted using aseptic technique, and the importance of aseptic technique should not be underestimated. It is designed to reduce post-surgical complications,

e.g., infections, wound dehiscence; improve animal survival rates and hasten return to the basal physiological functions that were present in the animal prior to surgery. Specific regulations and guidelines can be found in the Animal Welfare Regulations (AWRs) and the *Guide for the Care and Use of Laboratory Animals (Guide).*

Pre-Surgical Preparation of the Animal

- Ascertain the animal is properly anesthetized before starting procedures. This can be done by determining the response to a painful stimulus (I.e. toe pinch). In addition, observe how relaxed the animal is (check jaw tone) and check the palpebral response which can indicate depth of anesthesia. Heart rate, respiratory rate, and blood pressure are also useful parameters to determine depth of anesthesia.
- After anesthetic induction, administer eye lubrication, e.g., Puralube, to prevent the occurrence of corneal ulcers or other eye damage. The blink response is generally blunted but this varies by species and other reflex responses should be used.
- Provide a heat source or heat sources before beginning the surgical preparation of the animal.
- Analgesia (pain relief) is generally required and should be administered prior to surgery, or at minimum, prior to recovery to allow effective systemic levels to be obtained before return of consciousness.

Preparation of the Surgical Site

- Proper preparation of the surgical site, i.e., skin, involves a number of steps. First the site of incision should be defined and hair or fur removed from an area approximately 200% larger than the area of the incision, by clipping and/or by using a depilatory agent. Care must be taken to use depilatory agents sparingly and to remove any excess material to avoid chemical burns. All loose fur should be vacuumed or carefully dusted away in order to prevent translocation into the incision site. An Oster surgical clipper with #40 blade, or Oster Pro-Trimmer, is ideal for clipping hair or fur. Hair removal should be done immediately prior to the surgical procedure (not in advance).
- Once the surgical site is free of all fur, surgical preparation of the skin is performed. Either povidone-iodine (Betadine) or chlorhexidine scrub formulations are recommended as both agents have good bactericidal activity and contain a detergent (this is what differentiates scrub versus solution). The bactericidal activity of both agents are decreased by organic debris. Chlorhexidine may be preferred due to residual activity within the skin pores as well as less adverse skin reactions noted in many species. However, chlorhexidine is noted to be neurotoxic, ototoxic and cause ocular damage with contact. Using gauze squares (or a suitable alternative, e.g., cotton tip applicators or cotton balls), the area should be scrubbed beginning at the center of the incision site working out to the perimeter in a circular pattern. Another piece of gauze is selected, and the process is repeated for a minimum total scrub time of 3 minutes.
- The area is rinsed with gauze soaked in 70% isopropyl alcohol or 70% ethanol (sterile water or saline instead should be used for rinsing mucous membranes or open wounds). A final application of povidone-iodine solution or chlorhexidine solution or skin protection

film with an antiseptic (ChloraPrep or DuraPrep) may be applied prior to draping.

- Surgical draping aims to create a barrier that prevents microbial spread from the patient to the surgical field. Draping should be performed by the surgical team following surgical hand preparation and donning of a sterile surgical gown. Materials for draping should be resistant to penetration by fluids, should not tear with normal use and be able to be secured to the patient. The layers of draping may be dependent on the surgical procedure being performed. An initial layer generally consists of four drapes placed evenly around the surgical area. These drapes may be sterile towels secured by penetrating towel clamps or disposable drapes with adhesive strips. Once the towel clamps have been fastened, their tips are considered non-sterile. Towels should not be adjusted once placed as this will affect the mechanical movement of bacteria from the unprepared sites to the scrubbed area. A second layer of draping consists of a larger drape that covers all exposed portions of the patient which may extend to the instrument table to create a continuous barrier. The outer drape may be secured with non-penetrating towel clamps or adhesive materials. The second or final drape should be made of a water-impermeable material. Disposable draping materials are advocated to avoid staff exposure to potential blood borne pathogens. In addition, reusable cotton or muslin draping materials are not fluid impermeable and deteriorate with repeated washing.
- The goal of aseptic technique is to prevent the surgeon, the surgical site, instruments, implantable materials and equipment from becoming contaminated. Individuals who are not gowned and gloved should not touch or handle anything that has been sterilized. The surgeon should only contact the surgical site and previously sterilized equipment until the incision is closed.

Surgeon Preparation

Individuals participating in actual surgical manipulation, whether as the principal or assistant, must wear clean surgical scrubs (only worn in surgical area), masks, hair bonnets or caps, disposable shoe covers, sterile gowns and sterile gloves. Surgeons must prepare the skin of their hands and forearms (to the elbow) prior to gloving and gowning. Surgical hand asepsis can be performed by two different methods: water-based surgical scrub or scrubless, waterless surgical hand asepsis. The standard water-based scrub should follow these steps: open pre-soaked surgical scrub brush, wet hands and arms up to the elbows, use nail pick to clean nails under running water, wet sponge and squeeze to work up lather, wash each finger, hand, and arm to two inches above the elbows using the non-abrasive sponge for a minimum total scrub time of three minutes, then rinse hands and arms from finger tips to elbows, dry hands and arms with a sterile towel. The scrubless, waterless surgical hand prep using a wall-mounted dispenser of the hand antiseptic (Avagard) includes the following steps: apply to clean, dry hands and nails; for the first use of each day, wash hands and clean under nails with a nail stick; apply using a three-pump protocol as detailed by the signs near the Avagard stations; allow to air dry (do not use towels). Signs are provided detailing these steps near all DCM surgical scrub areas

Gowning and gloving are done with appropriate assistance in the operating room. A closed gloving technique, in which the surgeon gloves without allowing the hands to protrude from the sleeves of the sterile gown, is recommended. Sterile surgical gowns should be made out of disposable impermeable material and should be changed every 4 hours of the surgical procedure.

Cloth gowns are highly permeable to bacteria and degrade with repeated washing.

The steps in surgeon preparation are:

1. Change from street clothes into surgical scrubs.
2. Put on hair cover, mask and shoe covers (plus face shield or or other EHS approved shield in nonhuman primate surgery).
3. Scrub skin or use approved scrubless, waterless surgical hand antiseptic
4. Put on gown and gloves (both must be sterile).

The surgical team should be comprised of surgeon(s), anesthetist(s) and others as necessary. All individuals participating in surgery as advisors, observers, circulating nurses and anesthetists must wear surgical scrub suits, disposable shoe covers, masks and hair covers. Circulating nurses and anesthetists must wear non-sterile gloves. Hands should be clean but need not be scrubbed as described above.

Anesthetic Monitoring

a. Monitoring of animals during anesthesia determining the response to a painful stimulus. In addition observe how relaxed the animal is (check jaw tone) and check the palpebral response which can indicate depth of anesthesia. Heart rate, respiratory rate, and blood pressure are also useful parameters to determine depth of anesthesia.

b. Intraoperative monitoring recommendations are provided by species at the end of the chapter.

Anesthetic Recovery and Post-Operative Care

The postoperative period may be divided into three phases: recovery from anesthesia, between recovery from anesthesia and 72 hours postoperatively, and the subsequent interval to suture removal at 10-14 days postoperatively. The frequency of observation is determined by the condition of the animal and the nature of the procedure, e.g., a thoracotomy, in which a chest tube has been placed, requires more attention than a subcutaneous implant. As minimum guidelines, the following observation frequencies should be used:

Phase One - Recovery from anesthesia

- Endotracheal tube in place - observe continuously
- Lateral recumbency - observe every 20 minutes
- Sternal recumbency, non-ambulatory - observe hourly

Phase Two - Recovery to 72 hours

- Observe for signs of pain or distress every six to 12 hours, and re-dose analgesia as described in your CAC protocol.

Phase Three - 72 hours to 14 days

- Observe once or twice daily depending upon the procedure, medication frequency and other factors.

Particular attention during phases one and two should be focused on thermal support, hydration, nutrition, and analgesia. For more information on postoperative care, DCM will provide training.

Proper postoperative care must be adhered to for all surgeries. The incision must be examined daily for evidence of wound dehiscence or infection until it is completely healed.

Infection and abscess formation around skin clips is rare unless the routine care and husbandry of the animal is poor. Non-absorbable sutures and/or staples should be removed 7-14 days post-operatively, after which time the wound should have healed. Absorbable sutures may be left in some species with permission from DCM veterinary staff. Post-op incision care should be performed in accordance with the approved CAC protocol, unless an exemption is given by DCM veterinary staff.

It is the responsibility of the investigator, in consultation with the surgical veterinarian and animal care staff, to promote the optimal recovery of the animal from surgery and anesthesia by providing appropriate postoperative care. A number of factors, e.g., type of surgery performed, type and amount of anesthetic used, will modify the nature, duration and intensity of the postoperative care required in an individual animal.

DCM considers the postoperative period to be between the end of the surgical procedure and the removal of sutures or resolution of post-surgical complications. Postoperative care may only be provided by individuals with appropriate and relevant training. Although investigators are primarily responsible for providing postoperative care, DCM will monitor performance and ensure compliance with existing protocols. If instruction in postoperative care is needed, or if arrangements for care cannot be made with other members of the laboratory, investigators should contact DCM so consistent care is provided. DCM staff may intervene in any situation involving the care of an animal. Attempts are made to inform investigators when changes in care are instituted.

Analgesics should always be used in animals and this strategy for providing analgesia and assessing postoperative pain must be described by investigators in the CAC protocol. Investigators should be guided by the principle that procedures likely to cause pain in human beings are likely to cause pain in animals. Whenever possible, analgesics should first be given as premedicates. The same drug or agent with a different site of action in the “pain pathway” can then be given postoperatively. The intraoperative use of local anesthetics is also recommended as part of a total scheme to minimize the sensation of pain.

Non-Pharmacologic Methods to Reduce Pain

Supportive care of animals can hasten recovery in the post-operative period. Recommendations and considerations of supportive care include:

- Provide care of incision as needed

- Singly house until ambulatory and then place back with familiar group, if possible
- Use soft, absorbent bedding and nest material, provide surfaces that aid in ambulation
- Modify food and water access, e.g., provide food and water within reach
- Increase food palatability, e.g., provide moistened food
- Provide supplemental heat, e.g., a heating pad, and hydration SQ/IP (fluid therapy will depend on degree of dehydration, length of surgical procedure, and blood loss)
- Provide cryotherapy, e.g., cold packs, for acute inflammation
- Therapeutic heat can be used to reduce joint stiffness/muscle spasms after acute inflammation has resolved
- Provide physical therapy and massage if indicated

Species-Specific Responses to Pain

When assessing pain in animals, it is important to recognize species-specific variations. Some signs to look for to determine the level of pain control are given by species below:

Rabbits:

- Attempts to escape or hide, aggressive behavior if handled, reduced spontaneous (eating, moving, grooming, rearing) activity, complete immobility, altered gait, stretching, back arching, abdominal pushing or touching, sudden short movements during sleep, hunched up posture; piloerection
- Reduced appetite and water intake, excessive water consumption, excessive urination
- Excessive grooming or biting, lack of normal grooming, self-mutilation, soiling of the haircoat, accumulation of secretions around the eyes or nose, reduced body weight, reduced production of urine and feces, half closed eyes or orbital tightening, dull or unfocused vision, strained facial expression, bulging eyes, excessive respiratory rate or effort, lethargy, isolation
- Pointing of the nose, cheek flattening, whisker changes

Swine

- Abdominal splinting, reluctance to move, flank watching or kicking, repetitive lying down and standing, chronic recumbency, arching of the back, agitation, unreasonable calmness and tolerance to touch, assuming lateral recumbency for prolonged periods of time
- Grunting, forced expiratory effort, bellowing, crying, squealing, any vocalizations elicited by pressure in one or more areas
- Decreased appetite, lip smacking and curling
- Aggression in normally passive species, head pressing, teeth grinding, ear droop, eyes

dull and listless, reduced grooming efforts, lacrimation, blepharospasm, poor body condition, weight loss

Nonhuman Primates

- Hide pain well in presence of humans, usually need immediate attention if they look sick
- Loud and persistent vocalizations are rare
- General appearance of misery and dejection, huddled/crouched with head down
- "Sad" facial expression/grimace/glassy-eyed
- Avoids companions, stops grooming or becomes aggressive
- Abdominal pain through facial contortions, clenching of teeth, restlessness, and shaking accompanied by grunts and moans
- Head pain may be manifest by head pressing against the enclosure surface
- Self-directed injurious behavior may be a sign of more intense pain
- Usually refuse food and water
- Changes in response to familiar personnel or unwillingness to cooperate.

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8. Keating SC, Thomas AA, Flecknell PA, Leach MC. 2012. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. PloS one. 7(9):e44437.
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Recordkeeping

DCM requires that medical records be maintained for all surgeries on non-rodent mammalian species. **Investigators should consult DCM with questions or concerns regarding recordkeeping or postoperative care.**

Multiple Survival Surgeries

Multiple survival surgeries require scientific justification. See the CAC policy "Multiple Major, Minor, Multiple & Other Surgeries" for more information.

Non-Survival Surgeries

Non-recovery surgical procedures, which have been described in an approved CAC protocol, may be performed in a CAC-approved laboratory or in the dedicated surgical facilities within DCM. If performed in the DCM surgical facility, the procedures must be carried out using strict aseptic procedure. Also, it is strongly recommended that all surgical procedures, even if terminal and taking place in a research laboratory, be performed as aseptically as possible to minimize experimental variables that might influence experimental results, e.g., terminal septicemia. For non-rodent mammalian species, it is necessary to complete an "Animal Termination Form".

These forms must be returned to the 46NHP or E25 pharmacy/surgery offices.

Guidelines for Surgery in Rabbits

Successful surgical manipulations are dependent on assessment of animal health pre-operatively, maintenance of adequate and stable anesthesia, smooth recovery from anesthesia and post-operative care.

1. Pre-anesthetic assessment and planning: For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. Rabbits should be acclimated for a minimum of 72 hours for a non-recovery surgical procedure or 7 days for a survival surgical procedure. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere. Preoperative fasting is not necessary in these species and should only be done if justified and approved in the animal protocol.
2. Analgesia: Appropriate pain management is crucial for optimal postoperative recovery. Analgesics should minimally be provided for the time period outlined in the CAC protocol and then as needed. Make sure analgesics are given on time; recheck intervals should match when the next analgesic dose is due or at a minimum of every 24 hours after analgesic dosing stops. Recommendations by procedure are made in the following table:

Mild Pain (analgesia may need to last up to 24 hours)	Option 1: Local Anesthetic • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision
	Option 2: Single dose NSAID before procedure • Meloxicam injectable: 0.3 mg/kg SQ before procedure OR • Carprofen injectable: 5 mg/kg SQ before procedure
	Option 3: Buprenorphine-HCl • Buprenorphine HCl 0.01-0.05 mg/kg SQ or IM every 6-12 hours for 1-2 doses
Moderate Pain (analgesia to last at least 48 hours)	Option 1: Buprenorphine-Sustained Release (SR) and NSAID for at least 48 hours • Buprenorphine-SR 0.12 mg/kg SQ once (lasts up to 72 hours) AND • Meloxicam injectable: 0.3 mg/kg SQ every 24 hours OR • Carprofen injectable: 5 mg/kg SQ every 24 hours
	Option 2: Local Anesthetic and Buprenorphine-Sustained Release (SR) • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision AND • Buprenorphine-SR 0.12 mg/kg SQ once (lasts up to 72 hours)
Severe Pain (analgesia to last at least 48-72 hours)	Please consult DCM veterinarian

3. Anesthesia: Many anesthetic protocols exist for rabbits and specialized anesthetic protocols can be developed in coordination with DCM veterinary staff and must be on an approved CAC protocol. Anesthetic agents include injectable and inhalant anesthetics. In general, inhalant anesthetics are advocated over injectable anesthetics for major surgical procedures. Below are some recommended anesthetic protocols:
    a. Induction:
        i. Ketamine/Xylazine: ketamine 35 mg/kg and xylazine 5 mg/kg IM can be used for minor surgical procedures alone or as an induction agent for intubation and maintenance on anesthetic gas.
            - Xylazine should be reversed at the end of the procedure or if the rabbit has respiratory depression with atipamezole 0.5 mg/kg IM.
        ii. Propofol: Following restraint and placement of an ear catheter after application of EMLA topical anesthetic, rabbits can be induced with Propofol 10 mg/kg to effect IV prior to intubation and maintenance on anesthetic gas.
        iii. Mask induction with isoflurane is generally not recommended in rabbits unless the reasoning to perform this is justified in the protocol.
    b. Maintenance:

i. Isoflurane is delivered through a laryngeal mask airway (LMA) or endotracheal tube (preferred) at 2-3%. The MAC of isoflurane for rabbits is higher compared to other species (2.05%).

c. Intraoperative support and monitoring: Anesthesia records are maintained during surgical procedures and stay with the animal's medical record.

i. Use of advanced anesthesia monitoring of rabbits during surgical procedures is advocated. This includes pulse oximetry, end tidal carbon dioxide, ECG, respiratory rate and temperature monitoring. The depth of anesthesia should also be closely monitored with lack of response to surgical stimulus. Pinna, pedal withdrawal, corneal and palpebral reflexes may be evaluated to help determine depth of anesthesia.

ii. Thermal support is required for all surgical procedures as hypothermia will occur with all anesthetic protocols. Temperature controlled thermal pads are recommended and can be adjusted as needed.

iii. Fluid therapy: intravenous fluids are advocated for the majority of procedures with intravenous catheters placed in the peripheral ear veins. Balanced fluids such as LRS should be given at approximately 5ml/kg/hr depending on procedure.

4. Recovery and post-operative support: Animals should be kept warm through the use of heating pads and, if animal size permits, body temperature should be monitored and recorded until it returns to normal. Be careful during preparation of the surgical site to not get rabbits excessively wet, which would lead to hypothermia.

a. Animals recovering from anesthesia should be rotated from side to side every half hour until they are able to maintain sternal recumbency.

b. Adequate hydration and nutrition is necessary in the healing animal patient. The gastrointestinal tract of rabbits requires close monitoring of food and water intake as well as fecal production. Please contact the facility veterinary technician or veterinarian if you have concerns about the post-operative recovery of the animals. Analgesic delivery in the post-operative period should match what is on the approved CAC protocol.

Guidelines for Surgery in Swine

Successful surgical manipulations are dependent on assessment of animal health pre-operatively, maintenance of adequate and stable anesthesia, smooth recovery from anesthesia and post-operative care.

1. Pre-anesthetic assessment and planning: For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. Pigs should be acclimated at a minimum of 72 hours for a terminal surgical procedure and up to 7 days for a survival surgical procedure. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere. Preoperative fasting is generally necessary prior to general anesthesia to prevent vomiting during anesthetic induction. An overnight

fast of eight to 12 hours is generally sufficient.

2. Analgesia: Appropriate pain management is crucial for optimal postoperative recovery. Analgesics should minimally be provided for the time period outlined in the CAC protocol and then as needed. Make sure analgesics are given on time; recheck intervals should match when the next analgesic dose is due or at a minimum of every 24 hours after analgesic dosing stops. Recommendations by procedure are made in the following table:

Mild Pain (analgesia may need to last up to 24 hours)	Option 1: Local Anesthetic • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision
	Option 2: Single dose NSAID before procedure • Carprofen injectable: 4 mg/kg SQ before procedure
	Option 3: Buprenorphine-HCl • Buprenorphine HCl 0.05-0.1 mg/kg IM every 8-12 hours for 1-2 doses
Moderate Pain (analgesia to last at least 48 hours)	Option 1: Local anesthetic prior to incision and NSAID for 48 hours • Bupivacaine 3 mg/kg local infiltration prior to incision OR • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision AND • Carprofen: 4 mg/kg SQ or PO every 24 hours OR • Bup SR at 0.18mg/kg SQ OR • Buprenorphine HCl ER at 0.12-0.24mg/kg SQ (3 mg/ml or 10 mg/ml)
	Option 2: Buprenorphine-HCl for 24-48 hours and NSAID for 48 hours • Buprenorphine HCl 0.05-0.1 mg/kg IM every 8-12 hours AND • Carprofen: 4 mg/kg SQ or PO every 24 hours OR • Bup SR at 0.18mg/kg SQ OR • Buprenorphine HCl ER at 0.12-0.24mg/kg SQ (3 mg/ml or 10 mg/ml)
Severe Pain (analgesia to last at least 48-72 hours)	Please consult DCM veterinarian

ROUTES OF ADMINISTRATION- "SQ" means subcutaneously, "IP" into the peritoneal cavity, "PO" by mouth, "IM" into a muscle, "IV" into a vein.

DOSING INTERVALS- "SID" means once a day (every 24 hours), "BID" twice a day (every 12 hours), "TID" three times a day (every 8 hours), "QID" four times a day (every 6 hours), "PRN" as needed. Alternatively, dosing interval can be written "Q 8 h, i.e., every 8 hours".

3. Anesthesia: Many anesthetic protocols exist for swine and specialized anesthetic protocols can be developed in coordination with DCM veterinary staff and must be on an approved CAC protocol. Anesthetic agents include injectable and inhalant anesthetics. In general, inhalant anesthetics are advocated over injectable anesthetics for major surgical

procedures. Below are some recommended anesthetic protocols:

a. Induction: Atropine may be given as a premedicant (0.05 mg/kg IM) and every 45-60 minutes thereafter during surgery.
    i. Midazolam/Dexmedetomidine: Midazolam 0.25 mg/kg and dexmedetomidine 0.03 mg/kg IM can be used for minor surgical procedures alone or as an induction agent for intubation and maintenance on anesthetic gas. Note that anesthetic gas may need to be first delivered by face mask prior to intubation with this drug combination.
        - Dexmedetomidine should be reversed with atipamezole (0.1mg/kg, IM) to expedite recovery
    ii. Telazol/Xylazine: Telazol 5 mg/kg and xylazine 2 mg/kg IM can be used for minor surgical procedures alone or as an induction agent for intubation and maintenance on anesthetic gas.
        - Xylazine should be reversed at the end of the procedure or if the pig has respiratory depression with atipamezole 0.1 mg/kg IM.
b. Maintenance:
    i. Isoflurane is delivered through an endotracheal tube (preferred) at 1.5- 3%.
c. Intraoperative support and monitoring: Anesthesia records are maintained during surgical procedures and stay with the animal's medical record.
    i. Use of advanced anesthesia monitoring of swine during surgical procedures is advocated. This includes pulse oximetry, end tidal carbon dioxide, ECG, respiratory rate and temperature monitoring. The depth of anesthesia should also be closely monitored with lack of response to surgical stimulus as well as changes in heart and respiratory rates.
    ii. Thermal support is required for all surgical procedures as hypothermia will occur with all anesthetic protocols. Temperature controlled thermal pads are recommended and can be adjusted as needed.
    iii. Fluid therapy: intravenous fluids are advocated for the majority of procedures with intravenous catheters placed in the ear veins. Balanced fluids such as LRS should be given at 5-10 ml/kg/hr.

4. <u>Recovery and post-operative support</u>:
    a. Animals should be kept warm through the use of heating pads and, if animal size permits, body temperature should be monitored and recorded until it returns to normal.
    b. Animals recovering from anesthesia should be rotated from side to side every half hour until they are able to maintain sternal recumbency.
    c. Adequate hydration and nutrition are necessary in the healing animal patient. Please contact the facility veterinary technician or veterinarian if you have concerns about the post-operative recovery of the animals. Analgesic delivery in the post-operative period should match what is on the approved CAC protocol.

Guidelines for Surgery in Nonhuman Primates

Successful surgical manipulations are dependent on assessment of animal health pre-operatively, maintenance of adequate and stable anesthesia, smooth recovery from anesthesia and post-operative care.

1. Pre-anesthetic assessment and planning: For experimental surgical procedures, only healthy and acclimated animals should undergo anesthetic and surgical events. Pre-emptive analgesia is advocated for all surgical procedures and is covered elsewhere. Preoperative fasting is generally necessary prior to general anesthesia to prevent vomiting during induction. A minimum fast of four hours is generally sufficient.
2. Analgesia: Appropriate pain management is crucial for optimal postoperative recovery. Analgesics should minimally be provided for the time period outlined in the CAC protocol and then as needed. Make sure analgesics are given on time; recheck intervals should match when the next analgesic dose is due or at a minimum of every 24 hours after analgesic dosing stops. Recommendations by procedure are made in the following tables by species:

Marmosets:

Mild Pain (analgesia may need to last up to 24 hours)	Option 1: Local Anesthetic prior to incision • Bupivacaine (0.25-0.5%) 2-4mg/kg local infiltration • Lidocaine (1-2%) 2-4 mg/kg local infiltration • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration prior to incision
	Option 2: Single dose NSAID before procedure • Meloxicam injectable: 0.2 mg/kg SQ or IV
	Option 3: Buprenorphine-HCl (standard release) • Buprenorphine-HCl 0.005-0.01 mg/kg SQ, IM every 8-12 hours • Buprenorphine Extended Release formulations (ZooPharm) 0.12-0.2 mg/kg SC q48-72 hours o NOTE: Full or partial reversal with naloxone (0.1 mg/kg IM, IV – Repeat admin PRN based on clinical response), will need to supplement analgesic if reversed o Sustained release formulations are not reversed o Not to be given immediately pre-operatively due to increased risk of apnea with sedation
Moderate Pain (analgesia to last at least 48 hours)	Option 1: Local anesthetic prior to incision and NSAID for 48 hours • Meloxicam Single loading dose: 0.2 mg/kg SQ or PO once o Followed by 0.1 mg/kg every 24 hours • An oral meloxicam suspension allows for oral dosing beyond the first dose given SQ at the withdrawal of general anesthesia.
	Option 2: Buprenorphine-HCl (standard release) for 24-48 hours and NSAID for 48 hours

Severe Pain (analgesia to last at least 48-72 hours)	Consult a DCM veterinarian

Macaques:

Mild Pain (analgesia may need to last up to 24 hours)	Option 1: Local Anesthetic prior to incision • Bupivacaine (0.25-0.5%) 2-4mg/kg local infiltration • Lidocaine (1-2%) 2-4 mg/kg local infiltration • Combination lidocaine 2 mg/kg and bupivacaine 2 mg/kg local infiltration
	Option 2: Single dose NSAID before procedure • Meloxicam injectable: 0.2 mg/kg SQ • Carprofen (Rimadyl) 4.4 mg/kg SQ • Ketoprofen 5 mg/kg IM/IV
	Option 3: Buprenorphine • Buprenorphine-HCl 0.02-0.03 mg/kg SQ, IM every 8-12 hours • Buprenorphine Long Acting (Simbadol) 0.24 mg/kg SC every 48 hours; 0.72 mg/kg SC every 72 hours

Moderate Pain (analgesia to last at least 48 hours)	Option 1: Local anesthetic prior to incision and NSAID for 48 hours
	• Meloxicam Single loading dose: 0.2 mg/kg SQ or PO once
	  • Followed by 0.1 mg/kg every 24 hours
	  • An oral meloxicam suspension allows for oral dosing beyond the first dose given SQ at the withdrawal of general anesthesia.
	• Meloxicam-Sustained Release: 0.6 mg/kg SQ every 48 hours
	• Ketoprofen: 5 mg/kg IM
	Followed by 0.1 mg/kg of Meloxicam (SQ or PO) every 24 hours

	Option 2: Buprenorphine for 48 hours and NSAID for 48 hours
Severe Pain (analgesia to last at least 48-72 hours)	Consult a DCM veterinarian

ROUTES OF ADMINISTRATION- "SQ" means subcutaneously, "IP" into the peritoneal cavity, "PO" by mouth, "IM" into a muscle, "IV" into a vein.

DOSING INTERVALS- "SID" means once a day (every 24 hours), "BID" twice a day (every 12 hours), "TID" three times a day (every 8 hours), "QID" four times a day (every 6 hours), "PRN" as needed. Alternatively, dosing interval can be written "Q 8 h, i.e., every 8 hours".

3. Anesthesia: Many anesthetic protocols exist for NHPs and specialized anesthetic protocols can be developed in coordination with DCM veterinary staff and must be on an approved CAC protocol. Anesthetic agents include injectable and inhalant anesthetics. In general, inhalant anesthetics are advocated over injectable anesthetics for major surgical procedures. Below are some recommended anesthetic protocols:

   Macaques:

a. Induction:
    i. Ketamine 10-20 mg/kg IM
    ii. Propofol 2-8 mg/kg IV given slowly to effect after induction.
b. Maintenance:
    i. Isoflurane delivered through an endotracheal tube. Recommended concentration for isoflurane is 1.5-2%.

Marmosets:

a. Induction:
    i. Alfaxalone 5-12 mg/kg IM or IV
    ii. Ketamine 10-40 mg/kg IM
    iii. Both these agents can be given in combination with Midazolam (dosage varies)
b. Maintenance: Isoflurane is delivered through a mask or an endotracheal tube at 1.5-3%.

5. Intraoperative support and monitoring: Anesthesia records are maintained during surgical procedures and stay with the animal's medical record. Vitals are recorded every 5-10 minutes during the surgical procedure.
    a. Use of advanced anesthesia monitoring of primates during surgical procedures is advocated. This includes pulse oximetry, end tidal carbon dioxide, ECG, respiratory rate, non-invasive blood pressure and temperature monitoring. The depth of anesthesia should also be closely monitored with lack of response to surgical stimulus as well as changes in heart and respiratory rates.
        i. At minimum, heart rate, pulse oximetry and respiratory rate should be monitored during surgery.
    b. Thermal support is required for all surgical procedures as hypothermia will occur with all anesthetic protocols. Temperature controlled thermal pads are recommended and can be adjusted as needed.
    c. Fluid therapy: intravenous fluids are advocated for the majority of procedures with intravenous catheters placed in an extremity (cephalic, saphenous, or tail veins). Balanced fluids such as LRS should be given at 3-5 ml/kg/hr unless indicated by the surgical protocol.
6. Recovery and post-operative support: Animals should be kept warm through the use of heating pads and, if animal size permits, body temperature should be monitored and recorded until it returns to normal.
    a. Animals recovering from anesthesia should be rotated from side to side, every fifteen minutes to half hour, if safe to do so, until they are able to maintain sternal recumbency.
    b. Adequate hydration and nutrition is necessary in the healing animal patient. Please contact the facility veterinary technician or veterinarian if you have concerns about the post-operative recovery of the animals. Analgesic delivery in

the post-operative period should match what is on the approved CAC protocol.

i. Prolonged recovery is often a consequence of hypothermia; rectal temperatures must be monitored carefully.

ii. Recovery location:

- Macaques are typically recovered in their home cage. Marmosets are recovered in capture boxes or incubator. They should be returned to their home cage once fully recovered from anesthesia.

Guidelines for Surgery in Finches

Many of the surgical practices for small rodents can be applied to zebra finches.

Finch surgeons are required to attend a finch-specific surgery wet lab from DCM. The finch-specific surgery wet lab covers topics including: applicable anatomy and physiology; proper restraint and handling; animal positioning; different injection techniques; preparation for surgery; sterile gloving and draping; sterile instrument-tip technique; hot bead sterilizer use; instrument and tissue handling; anesthesia, analgesics; anesthesia monitoring; estimation of blood loss and hemostasis; closure; subcutaneous fluid therapy; recovery; postoperative monitoring; and euthanasia.

Finch surgeons are also required to take the Surgery/Post-Op Wet Lab e-course that covers aseptic technique, gloving and draping, anesthesia and analgesia, and post-op care. A Refresher e-course surgery training is required of all surgeons every three years. Consult a DCM veterinarian with specific questions.

Chapter 11: Transgenic Services

DCM's Transgenics Unit offers the services listed below. Procedures, fees and electronic request forms can be found on the DCM website (TouchStone required)". If requesting microinjection procedures to generate genetically engineered mice, be sure these mice are accounted for in your CAC protocol.

Embryo Transfer Rederivation

- Performed to rederive mouse or rat lines of unknown or unwanted health status into "clean" animals.
- Animals from outside institutions are held in quarantine and naturally mated or hormone primed, followed by embryo harvest.
- Embryos are transferred into known-health status recipient females in a clean facility; weaned pups are released when recipient female serology and parasitology are negative.
- The entire process takes at least 10 weeks.

Pronuclear Microinjection

- Performed to create transgenic mice by the introduction of DNA into the pronucleus of one-celled embryos.
- Usually involves C57BL/6NTac (Taconic) or C57BL/6J (JAX) donor embryos and a minimum of two CD-1 recipient females.
- Tissue samples for genotyping can be collected for the investigator(s). Pups are released once serology and parasitology on the recipient female are negative.

Lentiviral Microinjection

- Performed to create transgenic mice by the introduction of lentiviral vectors containing transgenes with or without microinjection into one-celled embryos.
- **It is necessary to have a Committee on Assessment of Biohazards-approved protocol to conduct this work. See https://cabescro.mit.edu/home**

Embryonic Stem Cell Microinjection

- Performed to create targeted mutants by injecting embryonic stem cells into blastocysts (diploid or tetraploid).
- Embryonic stem cells need to be tested for pathogens before microinjection.
- Several options exist for donor embryos depending on the strain of ES cells. Commonly used donor embryo strains include C57BL/6NTac, C57BL/6J, and BALB/cAnNTac.
- When pups are 7-10 days old, they are scored for coat color chimerism.

Cryopreservation of Embryos and Sperm

- Performed to transport or save mouse lines as an alternative to live animals and expansive breeding programs.
- Goal is to freeze/store/receive 250-500 embryos per line. All lines are stored in duplicate at MIT.
- C57BL/6NTac superovulated females are supplied or investigators can supply their own females and males, taking into account different responses to hormone priming.

In Vitro Fertilization

- Performed to salvage or expand lines of mice.
- Super-ovulated females are supplied and sperm are harvested from males of choice. Fresh or frozen-thawed sperm may be used.
- Once fertilized, the embryos are transferred into recipient females, as in ETR above.
- Weaned pups are released once serology and parasitology the recipient are negative.
- Laser-assisted and media-assisted in vitro fertilization is available.

Chapter 12: Comparative Pathology Services

Comparative Pathology Laboratory (CPL)

CPL is an integral part of the Division of Comparative Medicine (DCM) and has four primary functions:

1. To provide comparative pathology services in support of the veterinary care, quarantine and surveillance programs of the Division;
2. To make the services of the laboratory available to investigators within or outside MIT on a fee-for-service basis;
3. To participate in the research activities of the Division relative to animal care and disease control;
4. To participate in the formal and informal teaching programs conducted within the Division.

CPL is equipped to provide many "in house" technical services including microbiology (bacteriology, mycology, virology, parasitology), clinical pathology (hematology, clinical chemistry, urinalysis), molecular biology and serology. It also utilizes outside reference laboratories to further support its mission. CPL also incorporates a complete necropsy/histology service which includes the expertise of a board-certified veterinary pathologist.

For veterinary care (clinical/diagnostic cases), we do not accept requests directly from an investigator (see below for CPL Support to the MIT Research Community). Investigators

must contact a DCM veterinary staff member to arrange for CPL services for evaluating health problems in their animals. The clinical veterinarian, in consultation with the investigator, will determine the scope of the problem, initiate whatever corrective measures are required, and submit the appropriate samples to the laboratory for evaluation. This very important and mandatory chain of communication ensures that the veterinary staff is cognizant of animal health problems and precludes the inadvertent spread of infectious disease to other animals.

CPL Support to the MIT Research Community

The resources of the CPL are available to MIT investigators on a fee-for-service basis in support of their own research projects. In addition, DCM welcomes the opportunity for collaborative efforts. Individuals wishing to utilize CPL should contact the following groups to request services. Available services and fees can also be found on the DCM website.

- For Diagnostic Laboratory requests, contact: **Diagnostic Lab**
- For Necropsy/Histology/Pathology requests, contact: **DCM Pathology**

At present CPL does not offer routine necropsy services to investigators outside of DCM but can provide veterinary pathology expertise and training to investigators and/or lab members in tissue collection or other endpoints needed to perform their research.

It is important to make these arrangements well in advance, to ensure that the laboratory has the necessary materials, supplies and staffing to accommodate the request

Ovarian Transfer

- Performed to manage mouse colonies that are sub fertile.
- Involves removing ovaries from young females and transplanting into immunocompatible recipients.

Chapter 13: Security

Card Access

Unauthorized entry into DCM animal facilities may result in the interference of research activity, inadvertent exposure of trespassers to hazardous substances, transmission of infectious diseases to other animals within the facilities, or escape of animals. Vandalism and theft are major concerns. Unauthorized personnel, especially children, are strictly forbidden from entering the animal facilities. Permission and arrangement for tours through MIT/WIBR animal facilities are arranged with the DCM Director's office. Authorization for entry requires written approval by the PI and DCM's Access Authorization team (dcmaccess@mit.edu).

DCM must document any security breaches. While some security measures may seem to impede research activity, research personnel should recognize the need for such measures and cooperate fully.

Entrance into all animal facilities requires a computerized security card 24 hours a day. An individual animal room key or card access is necessary for entrance into animal rooms after normal work hours. Normal work hours are 7:00 am to 3:30 pm daily, weekends and holidays included. During working hours most animal rooms are unlocked except for areas with nonhuman primates, which require greater security. All MIT animal rooms are locked during non-working hours. WIBR policy is to keep animal rooms unlocked.

All facility access requests must be submitted to dcmaccess@mit.edu. In the interest of security, lost or stolen keys/cards should be reported immediately to dcmaccess@mit.edu. Unneeded cards and keys must be returned to DCM.

When Leaving MIT/WIBR

Personnel leaving MIT/WIBR are required to return keys and have access cards deactivated; Principal Investigators (PIs) are responsible for enforcement of this policy. You must inform DCM/CAC when you are leaving (dcmaccess@mit.edu; cacpo@mit.edu).

Who to Call in Case of an Emergency

a. The central phone number for DCM is 617 253-1757 and is answered by DCM 9 am-5 pm Monday-Friday. After hours, an answering service will route your concern to DCM.

b. For concerns about animals or animal facilities outside of normal business hours, a DCM veterinarian is available at 617-504-8375 or 617-304-5804; facility staff is available at 617 290-0597.

c. Fire or police actions - MIT Police - dial 100 from campus phone. WIBR emergencies are routed through Cambridge Police - dial 9-911 from inside WIBR.

- Emergency responses within DCM (based on scale of event) are always subject to-Institute responses which are led by the Cambridge Fire Department,

secondarily by MIT's MIT/WIBR's EHS (David Barber and Bill Van Schalkwyk).

- Not risking your safety or the safety of others.
- Always involve contacting a senior veterinarian to assess animal impact.
- **In all cases where research animals are put at risk by an emergency, DCM's Director (Dr Kelly Metcalf Pate, kpate@mit.edu) should be contacted as soon as possible after life-saving (i.e., medical emergencies) or building-saving (i.e., fire) contacts are made.**

d. You can access the emergency status of MIT from off-campus several ways:
   - MIT web home page and Emergency Information link (alphabetical index) or
   - Phone 617 253-7669 (3-SNOW).

DCM Emergency Plan

DCM has developed an emergency disaster plan entitled "Emergency Plan for the Animal Facilities at MIT and the Whitehead Institute", in the event that an emergency affects the research animal colonies at MIT/WIBR. The plan describes DCM's response, the institutional resources available, and the methods used to deal with the various types of emergencies that could occur. Some issues specific to employee safety are addressed, but the main focus is the provision of animal welfare and how to minimize animal-related losses to the research efforts at MIT/WIBR. This plan is intended to be subordinate to an MIT/WIBR-wide plan that comprehensively addresses employee safety and concerns of the general MIT/WIBR community.

Persons performing surgery or behavioral experiments should have a response plan to manage animals during fire alarms and other building evacuation orders. The following are general guidelines and if emergency responses are implemented, the specific event should be discussed with the facility veterinarian as soon as practical if there was any impact on animal welfare.

All laboratories with in-lab animal colonies must have a disaster plan on file with DCM and the CAC. Template for disaster plans are available from DCM.

Building Evacuation during a Surgery or Behavioral Experiments

As first priority, all staff need to ensure human safety and secondarily to minimize animal morbidity/mortality.

The courses of action outlined below are based on the procedure being conducted and the species involved.

8. <u>During Surgery of Non-Rodent, USDA-Regulated Species (e.g., guinea pigs, rabbits, ferrets, swine, nonhuman primates)</u>

a. Have a second person seek veterinary advice in the facility. If unavailable, call the PCS on-call iPhone, 617-504-8375, and/or the Veterinary iPhone, 617-304-5804, if neither is available call the DCM central phone number, 617-253-1757.

b. Close the incision, remove IV catheters, turn off anesthesia gas/oxygen, remove endotracheal tube, and return animal to a recovery cage in lateral recumbency on dry towels, cage board, or slotted rack.

    i. When positioned in the recovery cage, there must be sufficient space made to minimize chances of respiratory compromise.

    ii. Nonhuman primates can be positioned sitting up with squeeze back loosely in place to help support posture.

c. Turn off heat sources capable of overheating animals, i.e. heating lamps, electric heating pads.

    i. Controlled water recirculating blankets do not have to be turned off.

d. The decision to euthanize should be made by a veterinarian when feasible. If time does not allow for stabilization of the animal, the animal should be euthanized.

e. After evacuation, notify the incident commander that affected animals need to be checked by the researcher and Veterinary Staff as soon as possible.

f. When permitted back into the facility, check the animal and report its status to the DCM Veterinary Staff and Area Supervisor.

9. <u>During Surgery of Smaller Species (e.g., mice, rats, fish, frogs, birds)</u>

    a. Close the incision, turn off anesthesia/oxygen, and return animal to clean recovery cage (or fish tank) with access to water.

    b. If the surgery is being performed outside of the animal facility, do not attempt to return the cage to the animal facility.

    c. Turn off heat sources capable of overheating animals, i.e. heating lamps, electric heating pads.

        - Controlled water recirculating blankets do not have to be turned off.

    d. If time does not allow for stabilization of the animal, the animal should be euthanized.

    e. After evacuation, notify the incident commander that affected animals need to be checked by the researcher and Veterinary Staff as soon as possible.

    f. When permitted back into the facility, check the animal and report its status to the DCM veterinary staff and Area Supervisor.

10. <u>During Behavioral Experiments, Non-Rodent USDA-regulated species</u>

    a. Do not attempt to return animals to the animal facility.

        - Leave nonhuman primates in restraint chair with head unrestrained or transport box (as applicable) and access to fluids or fruit, if possible.

    b. After evacuation, notify the incident commander that affected animals need to be

checked by the researcher and veterinary staff as soon as possible.

c. When permitted back into the facility, check the animal and report its status to the DCM veterinary staff and Area Supervisor.

11. <u>During Behavioral Experiments of Smaller Species (e.g. rodents, birds)</u>

a. Do not attempt to return animals to the animal facility

b. Return animals to home cage with adequate food and water.

c. After evacuation, notify the incident commander that affected animals need to be checked by the researcher and veterinary staff as soon as possible.

d. When permitted back into the facility, check the animal and report its status to the

Chapter 14: Regulations and Guidelines for Animal Use in Biomedical Research

This section summarizes the laws, regulations, and guidelines for the use of animals in research and teaching as set forth by federal law and agencies, Massachusetts and Cambridge governments, and professional associations (AAALAC International and the American Veterinary Medical Association (AVMA)).

Federal

Animal Welfare Act (AWA) and Animal Welfare Regulations (AWRs)

The Federal law regulating the use of animals is the Animal Welfare Act (AWA) and its associated Animal Welfare Regulations (AWRs). The AWA, first enacted by Congress in 1966 and amended numerous times since then, mandates standards for transportation, care, and use of vertebrate animals used in research and covers all species, live or dead, except cold-blooded vertebrates, mice of the genus *Mus* and rats of the genus *Rattus* purpose-bred for research, and birds not bred in captivity. It also dictates the existence, structure, and function of Institutional Animal Care and Use Committees (IACUC), known as the CAC at MIT/WIBR.

The United States Department of Agriculture (USDA) is responsible for enforcing the AWA and for developing the AWRs. These latter consist of standards and record-keeping requirements affecting the purchase, handling, care, and use of laboratory animals. The standards include minimal requirements for restraint, housing, feeding, watering, sanitation, and ventilation; separation by species; veterinary care; appropriate use of anesthetic, analgesics and tranquilizing drugs; and nonhuman primate psychological well-being.

A USDA agent visits MIT at least once per year to assess compliance with the AWRs; the agent reviews CAC and DCM records and conducts inspections of DCM facilities and investigator laboratories where animal use occurs. These visits are unannounced. DCM files an annual report to the USDA to indicate the quantities of AWA-covered species that have been used in the prior twelve months and under which pain and distress categories they were used; the report must also identify any deviations from the regulations.

Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) and the *Guide for the Care and Use of Laboratory Animals* *(Guide)*

The Public Health Service (PHS) includes such agencies as the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA). **Any institution that receives PHS funding must provide formal assurance that use of vertebrate animals for research or teaching will follow the AWA and PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy) and adhere to the standards for animal welfare set forth in the *Guide for the Care and Use of Laboratory Animals* (*Guide,* 8th edition).** Unlike the AWA, PHS Policy applies to **all** vertebrate animals.

All laboratory animal users are strongly encouraged to familiarize themselves with PHS Policy and the Guide. Exceptions to the requirements in these documents require advance approval by the CAC.

The Office of Laboratory Animal Welfare (OLAW) is the division of NIH that exercises compliance oversight relative to PHS Policy. The CAC must inform OLAW annually regarding CAC membership and results of the semi-annual CAC facility and laboratory inspections that are required by the PHS policy. The CAC is also required to notify OLAW on a case-by-case basis of any serious or ongoing instances of noncompliance with the AWA, PHS Policy, and/or the *Guide*.

Massachusetts

The Commonwealth of Massachusetts has enacted a number of laws that impact the conduct of animal research within its boundaries, and there are specific regulations covering the use of various wild animal species, as well as cats and dogs (though these species are not housed at MIT/WIBR). The state imposes severe penalties for unauthorized access to animal facilities that results in willful and malicious injury or damage to or otherwise interferes with animals, data, equipment, or the facility.

Cambridge

In 1989, the City of Cambridge enacted an ordinance which requires the standards set forth by the AWA, PHS Policy, and the *Guide* be applied to all vertebrate animals without exception. A Commissioner of Laboratory Animals oversees the care and use of laboratory animals in Cambridge and is required to make at least one annual site visit to MIT to review CAC records and inspect animal and research facilities. The Commissioner also must approve the non- affiliated community member appointed to the CAC.

AAALAC International

AAALAC International is a nonprofit corporation of laboratory animal experts whose mission is to promote high standards of animal care, use, and well-being, and to enhance life sciences research and education through a peer review-based accreditation process. Participation in the accreditation program is voluntary at the request of individual animal programs. Full accreditation by AAALAC is recognized as assurance of the institution adhering to the principles mandated by PHS Policy, and continued accreditation influences funding for research.

An AAALAC team visits MIT once every three years to review our animal care and use program; MIT's animal care program has been accredited by AAALAC since 1972. DCM continues to strive toward meeting the rigorous standards set by this organization.

AVMA Guidelines on Euthanasia

The American Veterinary Medical Association (AVMA) is a nonprofit organization whose objective is "AVMA Guidelines for the Euthanasia of Animals: 2020 Edition" to advance the science and art of veterinary medicine, including its relationship to public health, biological science, and agriculture." MIT's policies are consistent with the "AVMA Guidelines for the Euthanasia of Animals: 2020 Edition."