

University at Buffalo
Institutional Animal Care and Use Committee

May 2021 Semi-Annual Report to the Institutional Official
of the Animal Care and Use Program Review and Facilities Inspection

TO: [REDACTED]

Executive Director; Regulatory Support FROM: Institutional Animal Care and Use Committee

SUBMITTED: June 7, 2022

This represents the semi-annual report of the Institutional Animal Care and Use Committee (IACUC) for the period of October 19, 2021, through May 16, 2022, as required by the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, Section IV.B.1.-3., the Guide for the Care and Use of Laboratory Animals (Guide), and the Animal Welfare Act (AWA) regulations, as applicable. Submission of semi-annual reports to the Institutional Official is a condition of this institution's Animal Welfare Assurance with the NIH Office of Laboratory Animal Welfare (OLAW).

Status of AAALAC Accreditation:

The University at Buffalo's Animal Care Program and Laboratory Animal Facilities has maintained Full Accreditation by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International during this reporting period.

Description of the Nature and Extent of the Institution's Adherence to PHS Policy, the Guide, and AWA:

The following protocols have been reviewed and approved by the IACUC and contain departures from the Guide, PHS Policy, and/or the AWA:

Fluid (Water) Regulation

- o PROT0000044 (rats): Water restriction is used as a positive reinforcement in the delay discount tasks
- o PSY14095N (rats): Water restriction is used as motivation for animals to sample the test stimuli or work for a water reward.
- o PSY2011 8Y (rats): Water restriction is used in the study of ghrelin's effects on regulating body fluids and fluid intake.
- o PROT02020000033 (mice): Water restriction is used as motivation for behavioral tests.
- o RIA06044N (rats): Water restriction is used as motivation for behavioral tests.
- o RIA13095Y (rats): Water restriction will occur prior to the experiment so as to normalize any pharmacokinetic measurements.
- o PROT0201800237 (rats): Water scheduling will occur during the pre-training phase for animals undergoing self-administration studies in order to provide motivation.
- o PSY03092Y (rats): Water restriction will be used to promote exploratory behavior and drug intake during testing periods.
- o PROT020190002 (Mice): Water restriction will be used as motivation for the behavioral test.
- o PSY17067N (rats): Water restriction is used in the study of Angiotensin II receptors and pathways in body fluid homeostasis.
- o ENS1086Y (rats): short water restriction prior to experiment to ensure all rats are in a similar acute hydration state.
- o ENS03077N (rats): Water is removed shortly prior to intake experiments to set up caging.
- o PROT0201900002 (rats): "water restricted Mice" will be trained to respond to changes in auditory stimuli.

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- o PROT0201900165 (rats): Water restriction is necessary to train rats to drink fluids during testing sessions and to motivate behavior.

Use of Wire-Bottom Caging

- o PROT0202000040 (mice): Metabolism cages are used to collect urine
- o PROT0202100085 (rats): Breeding pairs will be mated in wire-bottom cages so the presence of semen plugs can be detected. Breeding pairs will be mated in wire-bottom cages so the presence of semen plugs can be detected.
- o PROT0202000062 (rats): Breeding pairs will be mated in wire-bottom cages so the presence of semen plugs can be detected. Breeding pairs will be mated in wire-bottom cages so the presence of semen plugs can be detected.
- o PROT0201800104 (mice): Metabolic caging will be used for the collection of urine and feces and to measure food and water intake.
- o PROT0202000040 (mice): Metabolic caging will be used for the collection of urine
- o PROT0202000062 (rats): Breeding pairs will be mated in a wire-bottom cage so that the presence of sperm plugs can be visualized to determine successful breeding.
- o RIA07054Y (rats): Breeding pairs will be mated in wire-bottom cages so the presence of semen plugs can be detected.
- o PROT0202000011 (Chinchilla): large wire-bottom cages to accommodate a running wheel. Cages are also fitted with a plastic flooring for the area not covered by the running wheel.
- o PHC59019Y (mice): Wire-bottom caging will be used for the collection of urine and feces.
- o MED26095N (mice): Metabolic caging will be used for urine collection and to facilitate the measurement of daily proteinuria.
- o RIA10074N (rats): Breeding pairs will be mated in a wire-bottom cage so that the presence of sperm plugs can be visualized to determine successful breeding.
- o PSY17067N (rats): Hanging wire cages will be used for the measurement of food spillage.
- o PSY20118Y (rats): Suspended caging will be used for monitoring food spillage.
- o BCH04050N (rats): Breeding pairs will be mated in a wire-bottom cage so that the presence of sperm plugs can be visualized to determine successful breeding.
- o PROT0201800237 (rats): Wire-bottom caging will be used to study the effects of an impoverished housing environment.
- o BCH06064N (rats): Breeding pairs will be mated in a wire-bottom cage so that the presence of sperm plugs can be visualized to determine successful breeding.
- o BME04112Y (mice): Metabolic caging will be used for the collection of urine and feces.
- o CHE09018Y (mice): Metabolic caging will be used for the collection of urine and feces.
- o PHC03112N (rats) – Wire-bottom caging will be used to collect urine to determine the renal clearance of administered proteins.
- o ENS01086Y (rats): Wire bottom caging will be used for monitoring food spillage.
- o PSY14095N (rats): Wire-bottom caging is necessary to allow drainage tubing from the gastric fistula to exit the floor of the cage while allowing the animal to move freely.
- o PMY55061Y (rats): Wire-bottom caging will be used during Lickometer testing to ensure an electrical circuit is completed when the rat drinks from the water spout.
- o RIA06044N (rats): Wire-bottom caging will be used for monitoring food spillage and during Lickometer testing to ensure an electrical circuit is completed when the rat drinks from the water spout.
- o PSY03092Y (rats): Wire-bottom caging will be used during Lickometer testing to ensure an electrical circuit is completed when the rat drinks from the water spout.
- o PHC14093Y (mice, rats, ferrets): Wire-bottom caging will be used to collect chewed but unconsumed pica which will be weighed and recorded.
- o PED24116N (mice): Wire-bottom caging will be used to collect urine.
- o PED24116N (mice): Wire-bottom caging will be used to collect urine.

Non-Diurnal Light Cycle

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- o PMY35088N (mice): A 14-hour light/10-hour dark cycle will be used to determine whether melatonin or a melatonin agonist is able to modulate abnormal circadian rhythms induced by chronic exposure to certain drugs.
- o PMY36108N (mice): A constant dark, constant light, or 11-hour light/11-hour dark cycle will be used for 3 weeks, alternated with a diurnal light cycle for 3 weeks. The protocol studies circadian rhythm response to various stimuli and drug treatments.
- o PMY17095Y (mice): A 14-hour light/10-hour dark cycle will be utilized to disrupt circadian rhythms by splitting the activity rhythm into an endogenous rhythm and a light-entrained rhythm, which may be used as an alternative to suprachiasmatic nuclei lesions. Continuous running wheel access in either a 3.5-hour light/3.5-hour dark cycle or a 3-6 week constant-dark cycle will also be used to assess entrainment during behavioral tests.
- o PMY37108Y (mice): Mice may undergo a change from a 14-hour light/10-hour dark cycle to a 12-hour light/12-hour dark cycle for certain behavioral testing.
- o PSY12045Y (hamsters): Animals may experience 8-16 hour light cycles to mimic seasonal changes in day length. These fall within the normal range of annual day lengths for the species under study.
- o PMY18095Y (mice): Is approved for special and specific light-dark cycles to accomplish our experimental goals. These are necessary to study circadian rhythms
- o ENSO 1086Y (rats): reverse light cycle to facilitate measurements of food intake during lights-off.
- o PMY21047N (mice): Changes in the light-dark cycle are to test the drug's effects on circadian rhythms.
- o PROT0201800033 (mice): Day/night cycle should correspond with the seasons and be controlled by the lab.
- o PROT0201900039 (mice): Animals are exposed to changes in the light/dark cycle.
- o PSY11104N (rats): Animals will be housed in photoperiods that mimic seasonal changes in daylight

No Environmental Enrichment

- o PROT0202000044 (rats): Enrichment Waiver: to replicate previous housing conditions
- o PROT0202100060 (rats): Enrichment waiver: to prevent altered learning in rats
- o PROT0202100049 (rats): Enrichment waiver: to prevent altered learning in rats.
- o PSY07031N (rats): Enrichment waiver: Enrichment will not be provided in those experiments that are an extension of previous experiments in which enrichment was not used. Enrichment will be integrated into the protocol in a systematic way, as new lines of research begin.
- o PROT0201900002 (rats): Enrichment Waiver: During chronic noise exposure all objects are removed, and animals can hide behind to avoid being exposed to noise.
- o PROT0201900165 (rats): Enrichment Waiver: environmental enrichment can affect behavior as it relates to drug abuse.
- o PROT0201900203 (rats): Enrichment Waiver: Minimize influences on rat's behavior.
- o PMY35088N (mice): Enrichment Waiver: Enrichment may alter or affect behavior, with the exception of cages with breeding females who will be given enviro-dly one week before their anticipated due date. The enviro-dry will remain or be replaced in the cage until weaning.
- o PMY37108Y (mice): Enrichment Waiver: Enrichment provided to young animals may set up patterns of behavior carried to adulthood. It may affect circadian rhythms, which are under study.
- o PROT0201800237 (rats): Enrichment Waiver: The studies are aimed at comparing the effects of enriched and non-enriched environments on multiple endpoints.
- o PMY36108N (mice): Enrichment Waiver: Enrichment provided to young animals may set up patterns of behavior carried to adulthood. It may affect circadian rhythms, which are under study. Enrichment may also disrupt or interact with the movement of the running wheel for cages in which running behavior is being recorded.
- o PTH05028Y (rats): Enrichment Waiver: Enrichment has been shown to produce antidepressant-like effects, which may confound the study of mechanisms involved in antidepressant effects.

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- o ENS01086Y (rats): Enrichment Waiver: Enrichment items may be chewed or consumed, affecting the study of ingestive behavior.
- o ENS03077N (rats): Enrichment Waiver: Enrichment items may be chewed or consumed, affecting the study of ingestive behavior.
- o PSY03092Y (rats): Enrichment Waiver: For this drug self-administration study, enrichment has been shown to decrease drug-related phenotypes with great individual variability.
- o PSY20118Y (rats): Enrichment Waiver: Enrichment will not be provided in those experiments that are an extension of previous experiments in which enrichment was not used. Enrichment will be integrated into the protocol in a systematic way, as new lines of research begin.
- o PSY17067N (rats): Enrichment Waiver: Enrichment will not be provided in those experiments that are an extension of previous experiments in which enrichment was not used. Enrichment will be integrated into the protocol in a systematic way, as new lines of research begin.
- o RIA06044N (rats): Enrichment Waiver: A goal of the project is to investigate effects of environmental enrichment on behavioral measures of psychological traits thought to predispose individuals to drug addiction
- o PMY18095Y (mice): Enrichment Waiver: Enrichment has been shown to greatly influence the behavioral endpoints of our studies, therefore we limit enrichment to breeding purposes in our colony and require exemption for our experimental mice.
- o MED26095N (mice): Enrichment waiver: Enrichment can affect the diversity of the microbiome so it cannot be used for animals that are in certain stages of the experiment. Only the mice having experiments will need enrichment waiver, breeders does not need it. The duration is variable based on the experiment, and it is listed for each experiment.
- o PMY17095Y (mice): Enrichment Waiver: Enrichment has been shown to greatly influence the behavioral endpoints of our studies, therefore we limit enrichment to breeding purposes in our colony and require exemption for our experimental mice except where described as appropriate for experimental needs.
- o RIA01023N (mice): Enrichment Waiver: Enrichment profoundly alters synaptic function and exerts a confounding effect on eCB signaling.
- o PTH05028Y (rats): Enrichment Waiver: Enrichment has been documented to produce antidepressant-like effects, including inducing neurogenesis. As we are investigating mechanisms involved in antidepressant effects, enrichment would confound our experimental paradigm. However, we will continue to group-house the animals (n=2- 4/cage) as we have in the past.

Less Frequent Cage Changing:

- o PMY170095Y (mice): Cages are changed every 3 weeks to reduce the disruption of circadian rhythms
- o PMY18095Y (mice): When studying circadian rhythms of animals it is required to provide as little disturbance as possible to behavioral measurements, therefore they change cages every 2-3 weeks as necessary for experimental protocols and successful data collection.
- o MED26095N (mice): Mice are coprophagic and only need cage changes once per month.

Single housing:

- o PROT0202100084 (rat): Single housing is approved to avoid damage to the surgical area due to fighting.
- o PROT0202000071 (rat): Single housing is approved following surgery to avoid interaction to the surgical area.
- o PROT0202000084 (mice): Single housing is approved for breeding males to be removed from breeding cages.
- o PROT0202100060 (rat): Single housing is approved to measure food and fluid consumption.
- o PROT0202100041 (rat): Single housing is approved following surgery and thereafter if rats display any abnormal behavior. Single housing is approved for unforeseeable variables, such as fighting.
- o PROT0202100049 (rat): Single housing is approved following surgery to avoid interaction

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with the surgical site and to prevent social enrichment which alters rates of learning.

- o PROT0202100018 (mice): Single housing is approved for tumor-bearing mice will be housed singly, to prevent cage-mates from attacking tumors.
- o PROT0202100085 (mice): Single housing is approved to assess food intake.
- o PROT0202100089 (mice): Single housing is approved for tumor-bearing mice, to prevent cage-mates from attacking tumors.
- o PROT0202100061 (rabbit): Single housing is approved to prevent fighting.
- o PROT0202000011 (rats): Single housing is approved for measuring food consumption.
- o PROT0202000084 (mice): Single housing is approved for breeding males removed from breeding cages.
- o PROT0202100008 (rat): Single housing is approved after disease development.
- o PROT0202100021 (mice): Single housing is approved for animals with tumors to prevent cage mates from attacking tumors.
- o PROT0202000099 (rat): Single housing is approved for animals after pump implantation .
- o PROT0202100019 (mice): Single housing is approved for animals with tumors to prevent cage mates from attacking tumors.
- o PROT0202100045 (mice): Single housing is approved for breeding males removed from breeding cages.
- o PROT0202000100 (swine): Single housing is approved to prevent disrupting surgical sites.
- o PROT0202000101 (mice/ rats): Single housing is approved in the instance of fighting or illness.
- o PROT0202000034 (mice): Single housing is approved for measuring food consumption and after microdialysis probe implantation
- o PROT0202000040 (mice): Single housing is approved for urine collection in metabolism cages.
- o PROT0202000049 (mice): Single housing is approved to be used as a stressor in an experiment
- o PROT0202000071 (rats): Single housing is approved for the duration of the experiment to prevent issues with the jugular catheters
- o PROT0202000084 (mice): Single housing is approved to separate breeding pairs after mating
- o PROT0202000033 (mice): Single housing is approved for noise exposure experiment
- o PSY12045N (hamsters): Single housing is approve as hamsters are aggressive toward their cage mates. In these cases, it is necessary to separate them to prevent serious injury or death. Siberian hamsters are solitary in the wild. Hence, single housing is not a stressor for this species.
- o PROT0202000040 (mice): Single housing is approved for tumor inoculation
- o PROT0201900042 (mice): Single housing is approved for post-surgely and if fighting occurs.
- o RIA07054Y (rats): Single housing is approved after survival surgery to avoid damage to wound or implants by another animal and to separate breeding pairs after mating.
- o PTE23087N (rats): Single housing is approved post-surgery to keep cage make from damaging cannulations and minimize aggression .
- o PROT0201900203 (rats): Single housing is approved post implantation of jugular cannulations and harness ports.
- o PROT0201900031 (mice): Single housing is approved post-procedure.
- o PROT0201900073 (mice): Single housing is approved if fighting occurs.
- o PROT0201900047 (mice): Single housing is approved for breeding male removed from breeding cages.
- o PROT02020000012 (dog): Single housing is approved post-surgery.
- o PROT02020000033 (mice): Single housing is approved to control water intake and minimize social interactions .
- o PROT02020000034 (mice): Single housing is approved to ensure coJTect food consumption.
- o PROT02020000011 (rats): Single housing is approved to obtain accurate estimates of feed intake and an accurate estimate for voluntaiy wheel exercises .
- o PROT0202000049 (mice): Single housing is approved 5 weeks prior to behavioral tests.
- o PROT0201900078 (mice): Single housing is approved to separate breeding females.

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- o PROT0201900069 (mice): Single housing is approved post-surgery .
- o PROT0201900072 (mice): Single housing is approved for tumor bearing mice.
- o PROT0201800131 (chinchilla): Single housing is approved for single housing post procedure to monitor urine/ stool/ and food intake.
- o PROT0201900155 (rats): Single housing is approved for I week post-surgery.
- o PROT0201900168 (mice) Single housing is approved if fighting occurs.
- o PROT0201900153 (Dog): Single housing is approved following the creation of a stroke.
- o PROT020170002 (mice): is approved for single housing post-surgery.
- o MIC37048Y (mouse): Single housing is approved after inoculation.
- o HER34120N (rats): is approved for single housing if fighting occurs.
- o MED15083Y (swine): Single housing is approved in case an odd number of animals.
- o PSY10104Y (rats): Is approved for single housing since the objective of the study is to investigate the effect of single housing during the juvenile phase on social behavior and neural vasopressin
- o NSG08055N (rats): Is approved for single housing as some animals have a monitoring device mounted to them that could be damaged or removed by cage mates.
- o PHC26114Y, and PHC29035Y (mice): are approved for single housing because other animals in the cage can affect or damage the established tumor in the mice.
- o PHC34095N (rats): Is approved for single housing following insertion of microdialysis guide cannula to avoid damage to the surgical regions and cannula by other rats.
- o NSG01033N and NSG22112N (rabbits, rats, mice): Are approved to single house post-operative rats until fully recovered to prevent injury from cage mates.
- o RIA13095Y (mice, rats): is approved for singly housing rats to properly monitor food consumption and oral intake of certain drugs.
- o PSY07031N (rats): is approved for single housing of rats fitted with permanent guide cannula and catheters
- o RIA09064N (rats): is approved for single housing experimental rats post-operatively to avoid injury to the surgical area that might arise from social interactions. To eliminate any stress due to separation, the rats will be separated 2 weeks before the start of the study. Donor female rats will be housed separately after successful mating until we collect the amniotic fluid/placenta samples (21 days post insemination).
- o RIA1 1039N (rats): is approved for single housing to prevent cage mates from disturbing cannula/catheter implantations.
- o NEU02123Y and NEU07046Y (mice): are approved for single housing in the event that infected mice develop paralysis in order to prevent trampling by cage mates.
- o PHC59019Y (mice): is approved for single housing of mice while bearing xenograft tumors to reduce risk of injury caused by other mice and when receiving radiolabeled antibodies or proteins to protect other mice from radioactivity.
- o PHC24092Y (mice): is approved for single housing of mice following surgical implantation of cannula to protect the surgical area and following injections of cyclophosphamide.
- o PMY05072Y (mice): is approved for single housing of mice when injected with tamoxifen (biohazard).
- o PMY55061Y (rats): is approved for single housing following surgery to prevent cage mates from chewing or disturbing the surgical sites or implants.
- o PSY17067N and PSY20118N are approved for single housing of rats in order to monitor food/water/saline intakes.
- o PSY13056N (mice): is approved for single housing of mice involved in operant conditioning and preference experiments to maintain control over their environment including water intake and social experience.
- o PMY18095Y and PMY17095Y (mice): are approved for single housing of mice to allow for the recording of a single mouse's behavior in the behavioral testing procedures and to monitor food intake and their individual weights.
- o PMY41040Y (mice): is approved for single housing post-surgery to facilitate healing and reduce the risk of injury by other mice.

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- o PMY37108Y (mice): is approved for single housing of mice used in the sucrose consumption test in order to monitor their intake.
- o OPS02075Y (rats): is approved for single housing of rats following surgery in order to monitor the incision postoperatively to facilitate recovery.
- o PGY04053Y (mice): is approved for single housing of rats after stereotaxic injection of 6-OHDA to avoid damage to the surgical location by scratching or fighting.
- o PHC08128N (rats): is approved for single housing of mice after disease development to decrease distress caused by non-diseased developed animals. Single housing is also approved for animal pump implantation to prevent chewing on the device for up to 16 hours.
- o PROT0201700002 (mice): is approved for single housing post-surgery.
- o BCH29101N (rats): are approved for single housing of timed pregnant mice and rats.
- o PHC18034Y is approved for single housing of rats following chemotherapeutic treatment.
- o PMY49120N (mice, rats): is approved for single housing of rats following surgery to monitor postoperative recovery and to protect the cannula from cage mates.
- o PMY09073N (mice, rat): is approved for single housing post-surgery to prevent disruption of head caps. Single housing is also approved only if all other same-sex letter mates are heterozygotic.
- o BME04112Y is approved for single housing to facilitate the collection of urine and feces.
- o LAFO 1088N (mice/rats): Single housing is approved until the time of the rederivation procedure.
- o LAFI 0019N (mice/rats/hamsters): is approved for single housing of sentinel rodents
- o PSY03092Y (rats): is approved for single housing of rats following intracranial surgery or venous catheterization to protect the surgical incisions and implants.
- o CCEO 1112N (mice): is approved for single housing of mice when longitudinal studies are in progress to assist with identification of animals since these mice have clotting disorders that prohibit ear punches or tags.
- o CLS08085Y and CLS03064Y (rats): are approved for single housing of rats in which jugular catheters or intracranial cannulations have been implanted to prevent other rats from chewing or damaging the implants.
- o PHC43046Y (mice): Single housing is approved for mice receiving tumor cells will be "single housed". Since other animals in the cage can damage the established tumor in the mice, they will be housed individually.
- o BCH08076N (mice): Single housing is approved for mice within the breeding colony, so any breeder males temporarily removed from a female will be single housed, as well as, any male pups that are born without brothers.
- o PHC44086Y (mice): In a sub-set of studies, animals bearing subcutaneous xenograft tumors will be single housed to reduce risk for injury inflicted by other mice.
- o NEU09116Y (mice): If infected mice develop paralysis, they will be moved to individual housing in order to protect them from trampling. This housing will be maintained until the end of their life, as it is not anticipated that they will get better.
- o ENS01086Y (rats): Single housing is required to monitor food and fluid intakes.
- o PHC36105Y (mice): May need to be single housed when tumor progress until mice recovered or sacrificed.
- o PHC47017Y (mice): Animals receiving tumor cells will be "single housed". Since other animals in the cage can damage the established tumor in the mice, they will be housed individually.
- o NSG16037N (rats): Rats will undergo an invasive procedure to induce a stroke. These rats will have external sutures and can exhibit impairment as a consequence of the stroke. Therefore, it is best if they are kept separated.
- o OPS04097Y (rats): Animals will be individually caged post-op to allow for recovery from surgery and prevention of scavenging of surgical sites by cage mates until the conclusion of the experiment.
- o PHC55097N (mice): Mice may need to be single housed to prevent scavenging of tumors by cage mates when tumors enlarge (will consult veterinarians before singly housed) until mice

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recovered or sacrificed.

- o PHC56097N (mice): Mice may need to be single housed depends on health condition (when an unexpected health condition arises, the LAF veterinary staff will be notified. Mice will be singly housed if veterinarians justify single housing for individual health concerns) until mice recovered or sacrificed.
- o RIA16047Y (mice): The rats may be singly housed so that their ethanol and sugar intake can be accurately measured. If rats are implanted with either guide cannula or chronic electrodes, other rats may chew on them if group housed. In addition, on occasion male LE rats fight when group housed. These rats will be separated and singly housed if there is evidence of fighting. All other rats will be group housed.
- o ENS03077N (rats): Because dams will be single housed during pregnancy / parturition, all breeding animals will be singly housed other than mating. For animals undergoing behavioral testing (e.g., post-weaning) single housing is required to monitor food intakes.
- o BCH04050N (rats): Rats that are treated have to be single housed not to bias research results.
- o BCH06064N (rats): Single approved for rats housed plastic cage for pregnancy and lactation; single housed plastic cage for measurements of food consumption over a period of one week.
- o CLS01122Y (mice, rats): Singly housed for 1241 imaging studies, twenty days maximum.
- o MED02011Y (swine): Single housing approved for juvenile swine are group housed after surgical instrumentation. As they grow, they reach a size where their size necessitates them being housed one to a cage in the CTRC animal facility. At this time, they are also in experimental protocols (i.e. after the initial physiological study) and must be housed individually for scientific reasons as well as animal care concerns (e.g. to prevent other animals precipitating excess excitement, gnawing at catheters and surgical sites etc.)
- o BCH25016N (rabbit): Single housing approved to prevent aggression between male rabbits.
- o CLSO 1122Y (mice, rats): Single housing approved for 1241 imaging studies, for a maximum of 20 days.
- o MED04011Y (rabbits): Large adult rabbits will be received in shipments of 2. It is unlikely that rabbits will have been cage mates at the vendor. Male rabbits, in particular, are prone to fighting in an establishment of social hierarchy when first placed together. Females may also fight when not caged together for great lengths of time. Single housing avoids risk of (and distress from) fighting. Rabbits will be housed in the same room, and are not expected to reside in LAF housing for prolonged periods of time (which would allow for exploration of group housing options). Furthermore, it is imperative that rabbits be single housed in the period following survival abdominal surgery. Cage mates would be prone to scavenging abdominal incisions which increases risk of post-operative fatality. Since rabbits are housed short term and post-op single housing is mandatory, there may be some benefit to habituation to single housing upon arrival. Rabbits will be euthanized within 24 hours of surgery.
- o NSG31038N (swine): Animals will be singly housed following injury in order to reduce further trauma and/or removal of dressings by other animals
- o PSY14095N (mice, rats): Animals are single housed to monitor food and fluid intake.
- o CLS 15022Y (rats): Animals are single housed after surgery for a week. Rats that have been implanted with intracranial guide cannula must be singly housed to prevent other rats from chewing or damaging the implant.
- o PMY35088N (mice): Depending on the transgenic strain being bred, some pregnant females may require group-housing (maximum of 2/cage) while others may require single-housing. Certain transgenic strains may be more delicate than others and require housing that differentiates from normal requirements, with the best interest of pup survival to weaning as well as maternal female(s) survival through the breeding process.
- o PED10085N (sheep): Ewes will be housed alone in their stall, and let out as group, per LAF protocols.
- o CHE09018Y (mice): To determine route of nanoparticle elimination animals will be housed singly.
- o PMY36108N (mice): Mice are single-housed with a running wheel attached to Clocklab data collection system. The cages used are made specially to fit our running wheels and are too small

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to house more than one mouse comfortably. Additionally group housing would disallow for recording of a single mouse's behavior. Mice will not be regrouped once circadian tests are complete as this may result in fighting.

- o NEU06015Y (mice): After mice undergo microPET analysis and are exposed to 18F, they will be housed returned to dams for a minimum of 24 hours until 10 half-lives have expired. Non-nursing mice that are older than P21 will be housed individually.
- o MED19014Y (mice): Mice undergoing tumor removal will be single housed while the incision heals to prevent other mice in cage from damaging the surgical site.
- o PSY11104N (hamster): Female and male breeder hamsters can be housed together throughout pregnancy and lactation. However, it may be necessary to remove the male to prevent extra litters from being born. Adult males with sexual experience are often aggressive towards other males, and this prevents housing these males with others. When separated, breeder females will be housed with other colony females. If signs of aggression are noted, these females will be separated as well. Every effort will be made to minimize the number of animals needing to be single housed as well as the duration of single housing.
- o PTH03126Y (rats): Single housing approved for rats post stereotaxic surgery if fighting occurs. Single housing also approved for
- o NSG33119N (dog): Animals will require single housing post-operatively until recovered in order to prevent injury and allow for calm recovery.
- o PROT0201800237 (rats): Some animals are isolated and single housed in wire cages to investigate the effect of a deprived/low complexity environment. Pregnant dams are single housed because the food consumption is measured daily, birth process is monitored, and pups from different dams should never be mixed at birth.
- o PHC03112N (mice, rats): Animals will be single housed for the following reasons: (1) The collection of urine to characterize renal function will require the use of wire bottom metabolic cages, where animals will need to be housed individually over the 12-hour collection period. They will then be returned to their original cages. (2) To avoid post-operative scavenging of catheters and/or sutures.
- o PED24116N (mice): Animals will be single housed to allow for 24 hours of urine/stool collection.
- o PTE16082N (mice): The animals must be singly housed during specific study periods for breeding and to monitor individual food intake of respective research diets.
- o PHY02103N (rats): The protocol involves the use of a single pregnant female one at a time. The animal will be singly housed for a maximum of 24 hours.
- o PTH05028Y (rats): Animals will be single housed if fighting occurs.
- o PHY01051Y (mice): Mice which have been administered AAV intracranial by stereotaxic injection will be housed separately because of concerns over complications with the implanted optical fiber.
- o PMY57081Y (rats): Animals will be single housed if fighting occurs.
- o PHC14093Y (mice, rats, ferrets): For the rat pica feeding test, the animals will be singly housed during the testing period.
- o R1A06044N (rats): Rats must be singly housed so that the food and fluid intake measures are obtained only from single animals.
- o PSY12045Y (Hamsters): Hamsters will be single housed if fighting occurs.
- o MED26095N Single housing approved for mice to collect urine for 24 hours.
- o CCE01031Y (mice): For athymic mice, the animals are housed together upon arrival and singly after surgery in order to avoid animals biting or scratching other animals' bandages/wounds. For Cdh11-/- and the corresponding wild type mice, they need to be housed singly to monitor the mice activity in the night and their food consumption. In addition after surgery, they are housed individually to avoid animals biting or scratching on other animals' bandages/wounds. For Nanog inducible mice, they are housed together until surgery. Thereafter, they are housed singly to avoid animals biting or scratching on other animals' bandages/wounds.

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- o MED35026N (mice): Following mating, male mice will be singly housed due to aggressive tendencies after breeding. Mice will be housed singly for 7 days after thoracotomy to facilitate proper healing.
- o ORB10074Y (mice): Following mating, male mice will be singly housed due to aggressive tendencies after breeding.
- o RIA10074N (rats): Female rats will be singly housed while they are pregnant and while they are maintaining a litter to avoid any stress of separation around delivery and during maternal care. Females may be rehoused into groups after weaning and during periods of time they are not pregnant. Male breeder rats will be singly housed to avoid aggressive behavior that occurs when males are removed from their co-housed mate for any period of time.
- o NSG34010N (swine): Animals will require single housing post-operatively until recovered in order to prevent injury and allow for calm recovery.
- o PSY09094N (rats): Female rats will be singly housed while they are pregnant and while they are maintaining a litter to avoid any stress of separation around delivery and during maternal care. Females are housed in groups or rehoused into groups after weaning and during periods of time they are not pregnant. Male breeder rats will be singly housed to avoid aggressive behavior that occurs when males are removed from their cage-mate for periods of time (e.g., 1 week).
- o PROT0201700002 (mice): Animals will be singly housed after surgery to minimize the potential for scavenging (so scavenging does not happen by a cage mate).
- o PROT0201800071 (rats): Rats who undergo stereotaxic surgery for the implantation of a guide cannula and participation in the microdialysis procedure will need to be housed singly after surgery, until microdialysis for 2 reasons. 1) to protect the cannula from chewing/grooming by a cage mate and/or self, and 2) to acclimate the rat to single living as the microdialysis data can only be collected on one rat at a time. These rats will be adults (120 days of age at the start of the single housing).
- o PROT0201800101 (mice): Single housing approved for mice after ligation to prevent damage to pinnae.
- o PROT02018102 (rats): Single housing approved for post-surgery to prevent scavenging by cage mates.
- o PROT02018000131 (chinchillas) Single housing approved for chinchillas post-experiments to facilitate animal recovery.
- o PROT0201800237 (rats): Single housing approved for rats post-surgery to avoid damage to cannulations.
- o PROT0201900039 (mice): Single housing approved to monitor food consumption, activity and nest building.
- o PROT0201900040 (dog) Single housing approved to prevent injury and surgical site complications.
- o ORB15087Y (mice/rats): Single housing approved post procedure to prevent mice from scavenging pouches
- o PROT0201900002 (rats): Single housing approved for rats post-surgery for 5-7 days to facilitate surgical site healing. Single housing also approved for animals if aggression occurs among cage mates

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Deficiencies in the Institution's Animal Care and Use Program:

The IACUC performed the semi-annual program review during the fully convened IACUC meeting on May 16, 2022 using the Guide, 8th Edition and other resources (e.g. PHS Policy, AWA, and AVMA Guidelines for the Euthanasia of Animals) as a basis for the review. To facilitate evaluation, the committee utilized a template based on the Sample OLAW Program Review and Facility Inspection Checklist available on the OLAW website. No member was involuntarily excluded from participating in any portion of the review. The evaluation included, but was not limited to, a review of: (1) institutional policies and individual responsibilities; (2) IACUC membership and functions; (3) IACUC member experience and training; (4) IACUC records and reporting requirements; (5) husbandry and veterinary care (all aspects); (6) personnel qualifications (experience and training); (7) occupational health and safety of personnel; (8) disaster planning and emergency preparedness; and (9) security (personnel and facility). There were no program deficiencies noted during this reporting period. Details of the program review are provided in Attachment 1.

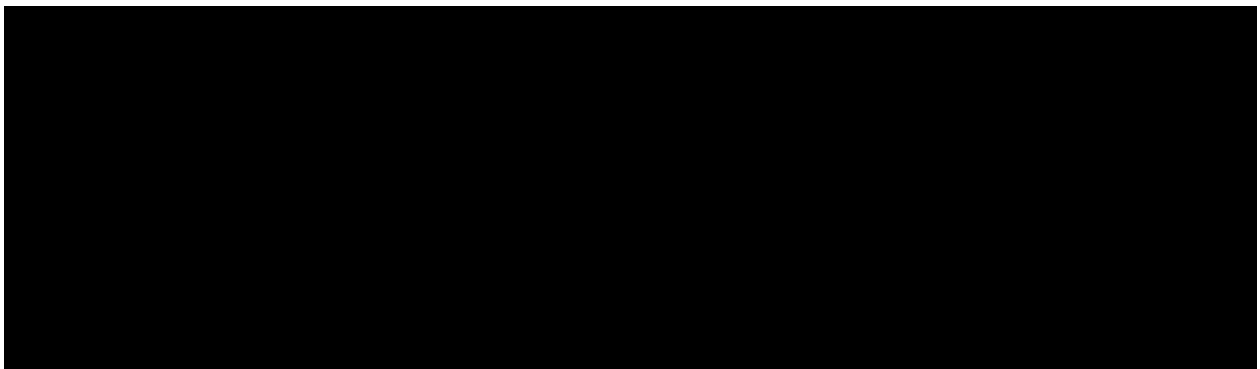
Deficiencies in the Institution's Animal Facilities and Investigator Laboratories:

The IACUC inspected animal facilities and investigator laboratories on March 23, 2022, March 25, 2022, March 28, 2022, March 30, 2022, April 5, 2022, April 7, 2022, April 11, 2022, April 13, 2022, April 15, 2022, and April 20, 2022 using The Guide, 8th Edition and other resources (e.g. PHS Policy, AWA, AVMA Guidelines for the Euthanasia of Animals) as a basis for the review. Documents and records maintained by the Laboratory Animal Facility which are relevant to the IACUC were reviewed on April 22, 2022. Members of the IACUC inspected any and all buildings, rooms, areas, enclosures, vehicles, and equipment used for animal confinement, transportation, maintenance, breeding, and/or experiments used for animal confinement, transportation, maintenance, breeding, and/or experiments inclusive of surgical manipulation. For areas where USDA-covered species are housed or used, at least two committee members were present during the inspection. To facilitate evaluation the committee utilized a template based on the Sample OLAW Program Review and Facility Inspection Checklist available on the OLAW website. No member was involuntarily excluded from participating in any portion of the review. The IACUC generally found the facilities and laboratories to be in good condition. No significant deficiencies were noted. A significant deficiency is defined as one which is or may be a threat to the health or safety of animals. Documentation of the schedule and participants for the inspections is provided in Attachment 2. A record of the minor deficiencies identified during the review, which includes reasonable and specific plans and schedules or correcting each deficiency, is provided in Attachment 3.

Minority Views:

No minority views were submitted or expressed by members of the IACUC.

Signatures of a majority of the IACUC members as required by AWR (§ 2.31, c, 3):



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Inspection Date	Repeat Deficiency	Location	Deficiency and Correction Action Needed	Responsible Party	Minor/ Significant	Due Date	Completion Date and Notes
3/23/2022	No		Rodent feces need to be cleaned from under the treadmill		Minor	4/8/2022	4/21/2022: The feces have been cleaned.
3/23/2022	No		Irradiator inspection sticker. The last inspection sticker indicated 2020		Minor	4/8/2022	3/31/2021: According to EHS records a radiation survey was last performed on this instrument on 6/26/2020 and is due to be surveyed again in 6/2022. The [REDACTED] lab was last inspected by Radiation Safety on 6/21/21 and will be inspected again this June.
3/23/2022	No		Did the room have microbiological testing within the last 12 months?		Minor	4/8/2022	4/8/2022: Microbiological testing has been completed.
3/25/2022	No		The vaporizer stickers were out of date. The lab stated that the company didn't place stickers on the machine this time around, but they would provide the IACUC with the documentation.		Minor	4/8/2022	3/25/2022: The vaporizer certification documentation was submitted it was very complete. It was recommended that the lab remove the expired stickers and keep the paperwork available for future inspections.
3/25/2022	No		There is a discrepancy in the drug log and drug left in "ket 145". There is a more significant amount of ketamine left in the bottle than indicated. The lab should check their math.		Minor	4/8/2022	4/22/2022: Per PI: The LAF staff got their numbers wrong. We used the correct amount and the amount remaining in the bottle is correct. They agreed with us after checking their calculations.
3/25/2022	No		Buprenorphine is expired		Minor	4/8/2022	4/26/2022: Buprenorphine has been returned to the LAF.
3/25/2022	No		Expired Fatal Plus		Minor	4/8/2022	4/25/2022: Fatal plus was returned to the LAF on 3/25/2022.
3/29/2022	No		Sunflower seeds need to be labeled with the expiration date and the PI's name		Minor	4/8/2022	4/4/2022: The sunflower seeds have been labeled with PI's name and date.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2021	3/29/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Expired powdered food needs to be removed from the room and the 8% CO2 machine needs to be cleaned.		Minor	4/8/2022	4/1/2022: The powdered food has been removed and the CO2 machine has been cleaned.
3/29/2022	No		The necropsy room needs to be cleaned		Minor	4/8/2022	3/29/2022: The necropsy room was cleaned on 3/30/2022

Attachment 3

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3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Did the room have microbiological testing within the last 12 months?		Minor	4/8/2022	4/22/2022: Microbiological testing has been completed.
3/29/2022	No		Did the room have microbiological testing within the last 12 months?		Minor	4/8/2022	4/22/2022: Microbiological testing has been completed.
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	4/22/2022: Microbiological testing has been completed.
3/29/2022	No		The room is very dirty and disorganized. Everything needs to be cleaned, organized, countertops need to be wiped down, floors need to be swept and mopped, the behavioral equipment needs to be thoroughly cleaned, and garbage needs to be emptied. Was RODAC testing completed within the last 12 months?		Minor	4/8/2022	3/29/2022: The room has been cleaned 4/21/2022
3/29/2022	No		Single housing stickers need to be placed on the cages		Minor	4/8/2022	3/31/2022: Single housing stickers have been placed on the cages.
3/29/2022	No		Expired items in the stereotactic surgical area		Minor	4/8/2022	3/29/2022: All expired items have been discarded.
3/30/2022	No		Did the room have microbiological testing within the last 12 months?		Minor	4/8/2022	4/26/2022: Room [REDACTED] has had microbiological testing.
3/30/2022	No		Did the room have microbiological testing within the last 12 months?		Minor	4/8/2022	3/29/2022: Behavioral rooms [REDACTED] and [REDACTED] have been tested in July 2021. No growth was observed.
4/7/2022	No		The feeding and watering chart needs to be completed daily. At the time of the inspection, it was only completed through 4/4/2022		Minor	4/27/2022	5/17/2022: Per PI proxy: I clarified this matter with animal care staff who checked with LAF Director. When the animals are fed, they are often fed for more than 1 day at a time. We now write "fed for X days" in the comment section of the log indicating the animals have been fed up to that date. Generally, animals are fed for 2 days at a time and then they receive a new disposable food container.
4/7/2022	No		Cages need single housing stickers and PI will feed and water cards need to be completely filled out		Minor	4/27/2022	4/22/2022: Single housing stickers will be placed on the cages.

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4/7/2022	No		Single housing stickers need to be placed on cages		Minor	4/27/2022	4/22/2022: Single housing stickers will be placed on the cages.
4/7/2022	No		Expired peanut butter needs to be removed		Minor	4/27/2022	4/22/2022: Peanut butter was discarded.
4/7/2022	No		Expired Marcaine and triple antibiotics need to be removed		Minor	4/27/2022	4/22/2022: All expired items have been removed.
4/7/2022	No		Steam and a high pitched squealing noise is coming out of the ceiling		Minor	4/27/2022	4/21/2021: This issue seems to be part of the building's HVAC and environmental control system. The issue is being rectified.
4/7/2022	No		Expired vetericyne was discarded. The lab needs to go through the cabinet and discard any additional expired items.		Minor	4/27/2022	4/20/2022: Any additional items were discarded.
4/7/2022	No		Expired items located in the cabinet in the room. Labs that share the room need to go through the cabinet.		Minor	4/27/2022	4/20/2022: The room was checked and any expired items have been removed.
4/7/2022	No		The strain cards need to be used for newly weaned animals. The lab was using "green cards" and wrote out the information		Minor	4/27/2022	4/20/2022: The PI will instruct their staff to use the new strain care for newly weaned animals.
4/7/2022	No		Single housing stickers need to be placed on cages		Minor	4/27/2022	4/27/2022: The lab has gone through the animal room and placed stickers on cages with missing stickers.
4/7/2022	No		The first aid kits contain multiple expired items. The blue cabinet needs to be gone through to ensure all expired items have been removed. All expired drugs and solutions need to be discarded. If a solution container has a formula please transcribe the formula and dispose of the used container. All solution containers expire 30 days after the solution is made and need to be labeled with the expiration date. All expired items need to be discarded from the refrigerator. The surgical tables need to be cleaned and organized, and the old chuck pads need to be discarded. What is the saline in the gallon bottle used for?		Minor	4/27/2022	4/22/2022: Per PI: Removal of expired items from first aid kits. Removal of expired items from the blue cabinet. Discard of all expired drugs. Transcription of container formulas. Discard of expired items from the refrigerator. Surgical tables cleaned and organized. Old chuck pads discarded. The saline is used for the collection of harvested tissue samples for histological analysis.
4/7/2022	No		Expired special diet needs to be removed from the refrigerator. All special diet needs to be labeled with expiration date and lab name. All secondary chemical containers need to have an EHS label. Please appropriately label all secondary chemical		Minor	4/27/2022	5/4/2022: The special diet has been removed and all new special diet will be labeled. All chemicals in secondary containers have EHS labels. All expired items have been removed.

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			containers. [REDACTED] has left a number of expired supplies, including doxycycline, please remove all of [REDACTED] supplies from the cabinets. Expired items can be donated to the LAF for training purposes.			
4/7/2022	No		Expired quick stop was discarded. A white lab coat was being stored in the blue cabinet.	Minor	4/27/2022	4/21/2022: Staff will look through the cabinet and dispose of any expired items. The PI will inform their staff that lab coats cannot be stored in the room.
4/7/2022	No		The drain needs to be flushed by running the sink water	Minor	4/27/2022	4/25/2022: Drain flushed
4/7/2022	No		Hood inspection is out of date. BSC-09-01. Serial # 72182	Minor	4/27/2022	4/25/2022: The hood has been inspected the sticker was not updated. A certification record was provided.
4/7/2022	No		Mouse Anesthesia- What is the percentage of ISO and does the lab use eye lube.	Minor	4/27/2022	4/28/2022: Per lab staff: Changes will be made to correct the issue regarding the lubricant. Anesthesia reports have not been submitted as I wasn't aware of this, this will be corrected as well. Regarding transport of the mice, mice transfer forms are submitted to relocate the mice to the [REDACTED] after IVT injections. The mice reside in the [REDACTED].
4/7/2022	No		Low humidity in rooms	Major		5/10/2022: The IACUC reviewed the issue on April 18, 2022, and decided it was an animal welfare concern. On 5/10/2022, an official IACUC letter was sent to the Director of Facilities.
4/13/2022	No		Animals in Slot G2 (ID # 254057 and 254058) have ulcerated tumors with necrosis and need to be euthanized as outlined in the protocols humane endpoints	Minor	4/27/2022	4/22/2022: Animals were euthanized
4/13/2022	No		The ceiling closest to the window is peeling	Minor	4/27/2022	5/16/2022: Facilities will be doing a large capital project to fix the issue and others in the building.
4/13/2022	No		The anesthesia area needs to be cleaned and organized. A number of expired items were found and need to be discarded.	Minor	4/27/2022	4/26/2022: The area has been cleaned and organized. Expired items have been discarded.
4/13/2022	No		Mouse Intravitreal injection: Lab staff didn't seem unclear regarding the details of the procedure. The IACUC is recommending the following points for the lab to focus on. The lab informed the inspectors that	Minor	4/27/2022	5/11/2022: Per PI: 5ul Hamilton Syringe is used for all the mice and not capillary glass needles. Hamilton is autoclaved prior to starting surgery and between each

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			capillary needles kept inside the large, plastic, petri dish were re-used between mice (after soaking needles in 70% EtOH). In fact, a new needle that is sterile before use should be used on each mouse. This means the needles should be autoclaved prior to use, then discarded after use. That did not seem to be what was happening. Reminder to apply Proparacaine (topical anesthetic) to the eye getting injected and allow 3-5 minutes to take effect. I don't recall them mentioning this step, but perhaps they did (?) Proparacaine needs to be kept refrigerated. Reminder to administer opioid analgesic buprenorphine at the conclusion of the procedure before the mouse wakes up from anesthesia. I don't recall them mentioning this step, but that doesn't mean they don't do it. A reminder that mice showing signs of ophthalmic pain after injections should get ophthalmic flurbiprofen (topical NSAID) drops, as needed. I don't recall this being mentioned. They should have this product on hand prior to starting any further injections, as it could be needed at any time. Reminder to monitor mice after the procedure for complications and to observe humane endpoints.			use the needle is disinfected with 70% ethanol. Refresh optive eye drops, 0.5% Proparacaine, 1% Atropine, and 0.3% Ofloxacin are applied every 3-4 mins in the listed order prior to intravitreal injection of the mice. Right after injection, buprenorphine diluted in PBS is also administered subcutaneously. All the mice seem to recover nicely back to normal. However, we will order flurbiprofen (topical NSAID) in case it is needed. Mice are monitored for at least an hour before returning them back to animal housing. 5/17/2022: The committee requested that the PI amend the protocol so the needles used are described and approved.
4/13/2022	No		The anesthesia vaporizer inspection is out of date.	Minor	4/27/2022	4/27/2022: The vaporizer will be inspected on 4/29/2022. The lab has an appointment with [REDACTED].
4/15/2022	No		Single housing stickers were missing from some cages	Minor	4/27/2022	4/21/2022: missing single housing stickers were placed on the cages.
4/15/2022	No		The drain needs to be flushed by running the sink water	Minor	4/27/2022	4/27/2022: The drains have been flushed.
4/15/2022	No		The room has a lot of PI equipment and no active protocol. The PI needs to clean out the room.	Minor	4/27/2022	5/16/2022: The room has been cleaned and organized. The floor is in bad shape Facilities will be addressing it after AAALAC.
4/15/2022	No		The behavioral chamber needs to be cleaned. Unknown expired fluid needs to be discarded. Expired food needs to be discarded. All expired items need to be discarded.	Minor	4/27/2022	4/21/2022: Chamber has been cleaned. The unlabeled fluid was 70% EtOH and has now been properly labeled. The expired food was discarded. The expired eye ointment was discarded
4/15/2022	No		The keys to the drug cabinet need to stay in a locked area and access to the drug cabinet needs to be restricted.	Minor	4/27/2022	4/21/2022: The keys were temporarily left with the student to allow the drug cabinet to be opened. The PI knew the

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						drug cabinet is empty and the key is normally locked up.
4/20/2022	No		The wood surface over which a behavioral device is kept will need to be covered with non-porous material. An induction chamber should be cleaned. The behavioral device should have disinfection validated per SOP 1.C.7 and a sign should be placed to make clear an anesthetic vaporizer that required re-inspection is not used.	Minor	5/3/2022	5/4/2022: The expired drugs have been returned, the induction chamber has been cleaned and the wood surfaces of the behavioral chamber have been covered by plexiglass. The lab is currently inactive microbiological testing needs to be completed when the lab becomes operational again.
4/20/2022	No		Nude mice (immunocompromised) are removed from sterile caging in an environment NOT within a biosafety cabinet/hood for laser irradiation under general anesthesia. However, being Nude mice, this breach in the sterile environment could predispose the mice to systemic infections. Steps taken to reduce likelihood of infection during this time as well as details (time out of sterile environment) should be added to the protocol (as well as scientific justification for why this must happen). Amend the procedure named Drug Activation by Laser Irradiation, Mouse. Ideally, cages should be labelled after mice breach the sterile environment and are returned to the Animal Holding Room. Materials that contact mice (tin foil laser guards) should be autoclaved between mice so they are sterile when placed in contact with mice. Induction chambers/nosecones should be disinfected in hood with high level disinfectant prior to using with Nude mice. Proper high level disinfectant was not previously used nor available in the hood on the date of inspection. Peroxigard is a recommended brand with a shorter contact time to be effective (1 minute). EtOH was presently in place in a properly labelled spray bottle (but this is neither a high level disinfectant nor sterilant per <i>The Guide</i> . EtOH is good to use on hood after work is done (to remove disinfectant residue to prevent erosion of surfaces over time). As explained to the IACUC, the hood is not turned on and left to run a full 5 minutes before being used. PI should review proper procedures for	Minor	5/3/2022	5/2/2022: Per PI: An arrangement for the isoflurane induction chamber and laser equipment has been made so that anesthesia and laser treatment can be performed without the nude mice leaving the hood. Images of this arrangement have been attached if the IACUC would like to review them. Going forward, mice will not be handled outside of the hood in [REDACTED], and all equipment including the tin foil laser guards will be sprayed with Peroxigard before being placed into the hood and are used with nude mice. The lab has placed an order for Peroxigard to be used for disinfecting the hood. The order confirmation for this has been attached. The SOP for Routine Hood Cleaning Procedure has been taped to the plexiglass of the hood and the phrases 'Please clean hood before and after use,' 'Turn on the blower and lights and allow it to operate for at least 5 minutes before using hood,' and 'Remove everything from inside the hood' have been highlighted. I also spoke with the only student currently working with nude mice regarding this, and suggested, if necessary, she reach out to the LAF manager to review proper technique of using immunocompromised mice inside of a Biosafety Cabinet.

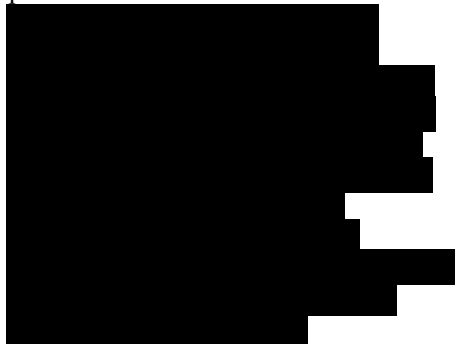
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			animal work within hood in [REDACTED] with all lab staff (though staff immediately committed to correcting the procedure, going forward). LAF SOP 4B23 should be followed https://www.buffalo.edu/content/dam/www/research/pdf/laf/sop/4B23.pdf . This is applicable for when the hood is used for immunocompromised mice (to avoid contamination).			
4/20/2022	No		Anesthesia machine is improperly connected to the induction box and has tape used to form seals between tubing/vaporizer. The green port of the Bain Circuit is pushed to connect directly to the vaporizer (versus proper use of tubing). Lack of proper adaptor pieces/well-fitting tubing would allow escape of waste gas to human users. Lab Staff was unaware of the requirement to apply sterile ophthalmic eye lubricant to generally anesthetized mice (indicated to us that this has never been done during anesthesia which is always very short, ~10 minutes). The anesthesia machine's precision vaporizer was directly connected to a large, yellow tank of "breathing air." This is different than 100% medical-grade oxygen available from the large green tank. A green tank was present, but empty and not connected to the anesthesia machine. 100% oxygen should be used for rodent anesthesia unless there is very specific approval in the IACUC protocol for "room air" or "breathing air" from a yellow tank. Lab Staff was unaware of the requirement to apply sterile ophthalmic eye lubricant to generally anesthetized mice (indicated to us that this has never been done during anesthesia which is always very short, ~10 minutes). Lab Staff was unaware of required validation of disinfection procedure needed for equipment that cannot be washed in cage wash. Mice are placed into a plexiglass holding chamber while under general anesthesia. This chamber is connected to electronic equipment and cannot be washed. Lab Staff correctly disinfects this with Lysol disinfectant wipes, but has never validated disinfection procedures per LAF SOP 1.C.7 (Sanitation of Research Testing Equipment). Advised use of commercially available induction box	Minor	5/3/2022	5/3/2022: The lab has ordered a new induction box and eye lubricant. They will return the oxygen tank and order a new one. The PI has advised the student to retake the Rodent Anesthesia. The lab will inform the LAF when they start a new experiment. The lab will follow 2. A. 26 Adult Rodent Anesthesia. The validation of research equipment needs to be completed before animal work starts again.

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			<p>to allow better connections with Bain circuit via standard adaptor pieces. (Bain circuit itself is okay and doesn't need replacement). Advised Lab Staff to take Rodent Anesthesia Module on UB Learns AND <u>retake</u> Rodent Anesthesia Wet Lab prior to ever anesthetizing mice again. (Staff did take this wet lab in March 2019.) Advised to have LAF Vet inspect anesthesia machine setup before ever anesthetizing mice again. Advised to apply sterile ophthalmic lubricant to eyes of mice upon removal from induction boxes (when placing them on nosecone for ultrasound procedure) per LAF SOP 2.A.26, Adult Rodent Isoflurane Anesthesia. Lab Staff and PI should review this SOP.</p> <p>Advised Lab Staff to acquire RODAC plates to perform validation testing but also explained LAF will not be placing a group order for plates until November 2022 (and does not currently have plates). Since no animal work is currently planned, this is another reason to close the protocol until animal work will be resumed.</p>			
4/20/2022	No		<p>Lab Staff explained their procedure for euthanasia (anesthesia with xylazine/ketamine) followed by exsanguination. However, staff explained "exsanguination" was completed via retro-orbital blood collection after general anesthesia was confirmed (though it was not yielding very much blood). RO blood collection is not an effective method of exsanguination, especially in a mouse under deep anesthesia. Exsanguination is generally accepted as cardiac puncture to allow a large volume of blood to be extracted quickly. Lab Staff explained cardiac puncture was not elected, as this could inadvertently cause lung puncture if done by traditional "blind stick" through diaphragm into left ventricle. There may have been confusion on what we meant by exsanguination. Until now (protocol is very new & animal work started last week), it is believe bilateral thoracotomy/lung removal as served as the secondary method of euthanasia. A discussion followed explaining thoracotomy to allow visualization of heart lungs and guided cardiac</p>	Minor	5/3/2022	<p>5/17/2022: Per PI: Response: We will perform exsanguination through cardiac puncture as suggested during the inspection. We will amend the IACUC protocol PROTO202100091 - Euthanasia:</p> 

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			puncture/blood collection prior to lung tissue harvest. Done well, this could prevent damage to lung tissue but still allow for large volume blood collection from the heart. The currently approved IACUC protocol, PROTO202100091, has a very non-specific procedure describing exsanguination <u>Euthanasia: Euthanasia by exsanguination, ver. (Team)</u> . The methodology and route are not well-described. The protocol should be amended to clarify this. Since thoracotomy may be used, an overdose of xylazine/ketamine (versus the currently approved anesthetic doses of 90/10 mg/kg ketamine/xylazine IP) should be considered to avoid breakthrough pain as ribs are cut. An overdose would be 2-3 times the anesthetic dose. Lab Staff may reach out to [REDACTED] for instruction on thoracotomy, cardiac blood collection and subsequent lung harvest.			
4/20/2022	No		Reminder was given to discard expired drugs (atropine-2019), though lab explained drug was not currently being used as they had switched to glycopyrrolate as anticholinergic. Lab Staff mentioned taking mice to [REDACTED] for imaging procedure in IVIS Unit in that room. However, later, neither the location of [REDACTED] nor the imaging procedure could be found on BME09055Y. Can PI explain where imaging is described in approved protocol (we may have missed it) and add [REDACTED] to Housing Section of protocol (Non-Vivarium Location Where Animals Used)? Or, are mice transferred to [REDACTED] protocol prior to imaging? A bottle of isoflurane was present in [REDACTED], and it was explained that this is used for the imaging procedure. Isoflurane is not found in the currently approved protocol. Only xylazine-ketamine injectable anesthesia is found. Which procedure is the Isoflurane used for? Static heat sources (gel bags) were found, but it was explained they are no longer used due to past problems. We asked that they be removed from the animal areas/discarded. The protocol does explain a "heating pad" is used while mice are anesthetized for Intratracheal instillations.	Minor	5/3/2022	5/20/2022 Per PI: we discarded the expired atropine-2019. EHS picked up the atropine-2019 on May 19. We also checked all drugs in our lab and make sure they are not expired. We will discard expired drugs immediately after the expiration date in the future. We imaged mice and their organs using the IVIS unit in [REDACTED] for our previous projects performed between 2017 and 2019. Since these projects were completed, they are not included in my current protocol. In the future, if we need to use the IVIS unit in [REDACTED] again for our projects, we will submit an amendment for this procedure and also add [REDACTED] to Housing Section of our protocol. Isoflurane was used in our previous projects performed between 2017 and 2019 to anesthetize mice for imaging procedures. Since these projects were completed, I removed them from my current protocol. My students and I went through our lab this morning and

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							found total 4.5 bottles of isoflurane left from these previous projects in our lab. The isoflurane was purchased in June 2018 and will expire in December, 2022. We transferred the remaining bottles of isoflurane to [REDACTED] lab. The gel bags were removed from the animal area.
4/22/2022	No		LAF SOPs 3. F. 4. Not listed in the binder		Minor	5/11/2022	5/2/2022: SOP 3.F.4 is listed in the table of contents.
4/22/2022	No		LAF SOPs 4. A. 33 Missing		Minor	5/11/2022	5/10/2022: The SOP is inactive and has been corrected in the SOP binder.
4/22/2022	No		LAF SOPs 4. B. 10 Missing		Minor	5/11/2022	5/10/2022: The SOP is current and has been placed in the binder.
4/22/2022	No		LAF SOPs 4. B. 15 2 of the same SOPs in the binder		Minor	5/11/2022	5/2/2022: The LAF took out the SOP dated/ Signed in 2017
4/22/2022	No		LAF SOPs 5. A. 7 Missing		Minor	5/11/2022	5/11/2022: The SOP is no longer active or relevant. It is been omitted.