University at Buffalo
State University of New York
Office of Research Compliance

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
MINUTES OF THE MEETING
February 21, 2022

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ATTENDANCE:

[redacted]

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CALL TO ORDER:

The IACUC Chair commenced the meeting at 12:32 PM.
If any member of the IACUC has submitted a protocol or amendment for review and approval, that member is not present during the discussion of and voting on their protocol or amendment. Quorum is maintained.

PRESENTATION OF THE MINUTES:
The minutes of the 2022 IACUC January meeting were presented. The January meeting minutes were voted on and unanimously approved.

BUSINESS:

Category E annual submissions: Submissions are listed under protocol review.

Annual Review for PROTO202000086
Annual Review for CCE02045Y
Annual Review for PROTO202000054
Annual Review for PROTO202000071
Annual Review for NSG08055N
Annual Review for MIC23035Y

BUSINESS:

Monitoring Charts: A monitoring chart review of NSG16037N: Monitoring charts were submitted for an experiment from March 202- to June 2021. The animals were monitored four times a day for the first forty-eight hours, and it was noted that several animals were found dead the following day. The committee recommends that the monitoring chart be modified to add a column indicating the time that the animals are checked. This is important during the first 48 hours of monitoring to ensure that the animals are monitored approximately every 6 hours.

PAM Update: The PAM site visit for 1/20/2022 has to be rescheduled. One of the students tested positive for COVID. The PAM officer will reschedule and complete this site visit, even if it is after the PAM officer's resignation date. The PAM officer position has been posted.

IACUC Policy Review: Policy on Review and Approval of Animal Use Protocols and Amendments. The IACUC no longer conducts an annual review of VA-funded protocols. The language regarding VA annuals needs to be removed from the IACUC policy, and USDA no longer requires annual review for IACUC protocols. The committee voted to remove annuals for USDA animal use protocols. The changes will be integrated into the policy and sent to the committee by email for approval.

Policy Review: The edited "IACUC Policy for Handling Issues of Noncompliance" was approved by email on February 4, 2022.

Possible Noncompliance: Monitoring chart review PROTO202000086. The monitoring chart was submitted in conjunction with AR202100093. Mouse 247079 reached a 21.7 % weight loss on 12/13/2021, and mouse 247080 reached a 35% weight loss on 12/14/2021. These animals were found dead on 2/17/2021. These animals were supposed to be euthanized at 20% weight loss per the monitoring chart. The monitoring chart indicates "con't supportive care for mice w/ wt loss" specifics of the supportive care were not outlined in the monitoring chart or the annual review details. The committee requested additional information from the PI to understand why the animals were allowed to reach a greater than 20% percent weight loss and were not outlined as per the protocol.

Noncompliance PROTO201900078: The committee reviewed the PI's response to the tail snipping noncompliance. The committee unanimously approved the PI's response, and the noncompliance issue will be closed.

Noncompliance PROTO2019001098: The committee reviewed the PI's response to the analgesic noncompliance. The PI did not provide the IACUC with a letter stating that they would ensure appropriate oversight and did not review and amend the protocol. The committee unanimously voted to require the PI to submit the appropriate documentation before the noncompliance issue can be resolved.

Semiannual Inspection: The semiannual Inspection will begin at the end of March. Committee members are to email the IACUC administrator to sign-up for the inspections.

Continued Training: ICARE Webinar was Wednesday, January 19, 2022. The biggest takeaway is that annuals for USDA animal use protocols are no longer required.

APPROVALS:
A list of the submissions approved since the last meeting has been presented to the committee.

PROTOCOL REVIEW:

In addition to IACUC review, Environment, Health & Safety (EHS) has also reviewed all protocols and amendments submitted this month. For protocols involving the use of hazardous agents in live animals, their use will be approved by the appropriate EHS authority and, as appropriate, laboratory SOPs will be placed as recommended by EHS prior to IACUC approval.

1. **AR202100093 (Annual Review for PROTO2020000086)**
   Annual Summary: From PI's annual summary: Over the last year 110 animals have been used in different experiments. For the first pilot experiment (9 mice, female) it was observed that the mice were not developing the adverse effects after 5-FU administration, including significant weight loss or changes in the oral mucosa after histological analysis, consistent with oral mucositis. For the second experiment (9 mice, female), the 5-FU drug was prepared using different solvents to test whether drug preparation was responsible for the lack of phenotype. However, no major adverse effects were observed. Histology analysis also did not yield the expected results. Based on these findings the protocol was amended to allow for an initial bolus of 5-FU drug at a higher dose with continued drug administration, anticipating this higher dose exposure will produce the expected effects. Prior to the next experiment, we collected tissues from a control group (5 mice, female) so as to have a comparison. For the third experiment (16 mice, female) the new drug administration protocol was utilized. A weight loss consistent with 5-FU toxicity was observed. Supportive care as described in our protocol guidelines was administered and no mice needed to be removed from the study prior to their end time points. Histological analysis showed changes in the oral and small intestine epithelium consistent with oral mucositis. For the fourth experiment (64 mice, female) there was an issue with the 5-FU drug being used and no effects were observed. For our most recent experiment (7 mice, male) two of the seven mice were found dead the morning of the day they were due to be sacrificed. All other mice were sacrificed on schedule and we were able to see significant weight loss which is consistent with the model we are aiming to achieve. Histology has not yet been done on these samples. Two mice found dead on 12/17/21, the day they were being planned to be sacrificed. Prior to this event mice were monitored daily (weighted and checked for body condition). From the moment mice reached 10% weight loss (12/12/12), lab staff started supportive care including wet chow and subcutaneous saline injections. Lab staff also communicated via email with LAF staff (on 12/12, 12/14 and 12/15), who indicated mice appeared in good condition. Despite the appearance of "good condition" mice were found dead, and should have been sacrificed earlier, as they had already reached the end-point.
   Upon necropsy, evidence of profuse bleeding was observed in the abdominal cavity.
   Committee Discussion: During the review of the monitoring charts, the committee noted that two mice were allowed to exceed the 20% weight loss threshold. The committee requested an explanation for why two mice were allowed to reach a greater than 20% weight loss. The committee requested the PI submit an amendment to increase the weight loss threshold.
   Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

2. **AR202100094 (Annual Review for CCE02045Y)**
   Annual Summary: From PI's annual summary: Things have been very slow, in fact, we've not done any work yet. But it's important to keep this protocol active as we have ongoing collaborations and pending proposals that could re-ignite research activity at any time.
   Committee Discussion: No category E animals were used in the past 12 months.
   Committee Action: The committee unanimously voted to approve the annual submission.

3. **AR202200002 (Annual Review for PROTO202000054)**
   Annual Summary: From PI's annual summary: Used 280 mice, do not see any unexpected results during my experiment.
   Committee Discussion: The committee requested the PI clarify the labs animal monitoring process and more details need to be added to the early removal of animals from the experiment. The committee also requested that animals be monitored for the duration of the experiment.
   Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

4. **AR202200012 (Annual Review for PROTO202000012)**
   Annual Summary: From PI's annual review summary: given the struggles of COVID and the global pandemic we have made strides to complete these aims and studies. We have used only a minimal number of animals for our studies. There have been no issues regarding animal health or well being
   Committee Discussion: No category E animals were used in the past 12 months.
   Committee Action: The committee unanimously voted to approve the annual submission.

5. **AR202200014 (Annual Review for MIC23035Y)**
   Annual Summary: From PI's non-scientific summary: We identified GRA15 as a Toxoplasma encoded seizure inducing gene. We also identified 3 other candidate genes as seizure inducing genes and began to test those as well as, in total we used 170 mice. All were category e
   Committee Discussion: The committee requested that the PI submit their monitoring charts to the IACUC or submit a copy, instead of only leaving them in the animal room.
   Committee Action: The committee unanimously voted to approve the annual submission.

6. **AR202200015 (Annual Review for NSG08055N)**
   Annual Summary: From PI's annual summary: Animals used in 2021:
   Overall we used 465 rats across 3 studies: 1. Short-term Morris water maze study: 94 rats total, 47 rats included (sufficient severity of TBI), 46 rats excluded (insufficient severity of TBI), 1 rat died from injury/surgery. 2. Moderate TBI injury with progesterone treatment: 32 rats total, 28 included in the study, 4 died from injury/surgery. 3. Therapeutic window study of NTS-104: 386 rats total, 111 included (sufficient injury severity), 135 excluded (insufficient injury severity, 140 died from injury/surgery.
   Committee Discussion: The committee reviewed the monitoring chart and noted that rats were found dead. The committee requested additional monitoring time points be added to the monitoring chart.
   Committee Action: The committee unanimously voted to approve the annual submission.

7. **PROTO202200002**
   Protocol Summary: From PI's non-scientific summary: Increased brain iron content coincident with blood-brain barrier degradation is an emerging concept of molecular disease manifestations in many neuropathologies. This protocol aims to assess brain iron loading using Perls and Turnbulls stains to detect two different oxidation states of iron, as well as Evans blue to assess vascular degradation. Investigation of this premise in a Friedreich's Ataxia mouse model will identify new targets of molecular disease, for which there is brain iron accumulation and aberrant vascular dynamics found in the human pathology.

Committee Discussion: Edits and clarifications need to be made in Click. NPG forms need to be added; any substances that are not pharmaceutical grade need to be scientifically justified. The use of CO2 for euthanasia needs scientific justification; the committee recommends deep anesthesia followed by a secondary euthanasia method. The needle size for "Evan's blue extravasation" needs to be stated. Perfusion should not be a stand-alone experiment, and it may unnecessarily increase animal numbers. The PI must state if the phenotype will potentially cause animal welfare issues.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

8. **TR202100049 (PMY41040Y)**
Protocol Summary: From PI's non-scientific summary: Specialized cells in the nervous system, called oligodendrocytes, extend fatty processes; known as myelin, that wrap neuronal projections, or axons. Myelin insulates the axons, so that electrical impulses are conducted down them more efficiently. A broad range of diseases, from congenital leukodystrophies to multiple sclerosis, result from myelin injury or loss. The aim of this work is to identify which transcription factors and signaling pathways regulate the induction of oligodendrocyte cell fate, the function of oligodendrocyte progenitor cells and other myelinating glia. We will also be determining whether new therapeutic drugs can improve myelin repair.
Committee Discussion: Edits and clarifications need to be made in Click. The emergency contact list needs to be updated. Any animals that undergo ear tagging genotyping or are being euthanized for tissue collection need to be listed as pain category C. The experiment "Modification of extracellular sulfation to improve oligodendrocyte differentiation in development and following demyelination (NMSS grant)" and "Determine the cellular identity of non-neuronal acetylcholine release during CNS demyelination" need clarifications regarding if animals will undergo multiple surgeries. The experiment "Modification of extracellular sulfation to improve oligodendrocyte differentiation in development and following demyelination (NMSS grant)" needs a husbandry exception for supplemented water. The experimental description for "Stereotaxic administration of lysolecithin to mouse corpus callosum" needs edits aseptic preparation, the experiment needs multiple substance administrations, and stereotaxic surgery needs to be a pain category D. The experiment "Quantification of neuronal ACh release through microdialysis" needs edits and clarifications to cannulation and microdialysis timeline. The experiment "Quantification of neuronal ACh release through microdialysis" and "Neuronal sulfatases" needs a husbandry exception for a special diet. The procedure "Topical analgesia for tail biopsy (>21 days)" needs a typo corrected. The procedure "Euthanasia: Sacrifice for tissue dissociation" and "Spinal Cord Injection-Surgery Drugs" needs edits to the Fatal Plus dosage, and needle size needs to be indicated. The procedure "Experimental substances are given during remyelination" needs details added for PI-88, IP injection dosage, and a substance administration needs to be added for supplemented water. The procedure "Mouse Spinal Cord Injection" needs edits to surgical preparation, suture removal, analgesia, and a monitoring procedure need to be added.
The committee recommends cold sterilant silk sutures for the procedure "Mouse Stereotaxic Surgery" the committee also requested clarifications to surgical prep and local anesthesia during the procedure. The animal justification page needs clarifications to the animal counts for each pain category. The alternatives section needs to be completed, and an emergency contact list must be added to the protocol.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

9. **TR202100051 (PROTO201800240)**

Protocol Summary: From PI's non-scientific summary: Monoclonal antibody technology was developed by Köhler and Milstein in 1975. This methodology immortalizes antibody-producing B cells through fusion with tumor cells. While the method is straightforward, their work was a major breakthrough as for the first time it allowed the unlimited production of a specific antibody molecule. The diagnostic and therapeutic implications of monoclonal antibodies were immediately recognized. In addition to important tools in basic research, these antibodies are now used in diagnostic tests, identification of novel drug targets, identification of vaccine antigens, biologic markers, and many more areas of research and development. In addition, now that these antibodies can be humanized, they are now used as therapeutic agents for the treatment of many different cancers as well as other diseases. This long-standing project is dedicated to the development of monoclonal antibodies to various antigens expressed by both Gram-negative and Gram-positive human pathogens. In addition this project provides this service to investigators that need specific antibodies developed in order to support their current research projects as well as supporting their continuing attempts at obtaining extramural funding.

Committee Discussion: The protocol was well written and complete.

Committee Action: The committee unanimously voted to approve the protocol.

10. **TR202100053 (MED03043Y)**

Protocol Summary: From PI's non-scientific summary: Moraxella catarrhalis (Mcat), a Gram-negative bacterial mucosal pathogen, is a significant cause of middle ear infections (otitis media (OM)) and sinusitis in infants and children and an important source of exacerbations in adults with lung disease. Approximately 80% of children under 3 will experience at least one episode of OM and many experience recurrent disease, which may result in hearing impairment and developmental/learning problems. OM is a significant source of direct and indirect health care costs and Mcat is responsible for 3 to 4 million pediatrician office visits annually. Chronic or recurrent OM is now considered a biofilm disease and there have been numerous reports demonstrating that Mcat forms biofilms in vivo. Biofilms on host surfaces promote adherence and persistence on mucosal tissues, provide protection from host defenses and antibiotics, and represent a primary source of chronic disease. Importantly, multiple studies have suggested that nasopharyngeal colonization with Mcat promotes subsequent persistent colonization with the two other major otopathogens Streptococcus pneumoniae (Spn) and/or nontypeable Haemophilus influenzae (NTHI), resulting in a significantly increased risk of OM.

1. The first goal of this proposed research project to evaluate whether Mcat facilitates colonization and persistence of the other otopathogens on the nasopharyngeal mucosa leading to increased incidence of OM and determine whether antimicrobial photodynamic therapy warrants further evaluation as a novel, efficacious therapeutic against chronic OM.
2. The second objective of this project is to develop monoclonal antibodies (MAbs) to novel adhesins involved in biofilm formation and stability as biofilms prolong infections in children and lead to multiple episodes of middle ear disease. These antibodies will be used to determine if the adhesins have potential as vaccine antigens or if these proteins can be exploited as novel drug targets. This is a critically important research area to the NIH/NIDCD as there is now a special emphasis on vaccine antigens and disruptions of biofilm. Novel therapeutic approaches designed to eradicate planktonic and biofilm-associated bacteria from the middle ear would not

only significantly decrease the constant use of antibiotics in young children, it would also decrease the associated morbidity and the need to perform surgeries for insertion of tympanostomy tubes.
Committee Discussion: The protocol was well written and complete.
Committee Action: The committee unanimously voted to approve the protocol.

11. TR20220001 (PMY09073N)
Protocol Summary: Taken from the PI's non-scientific summary: We have identified a novel mammalian neuropeptide system in the brain, i.e. a transmitter system using a peptide as the diffusible neurotransmitter (= ligand) that activates a specific receptor. Based on its primary structure the ligand has been termed Neuropeptide S (NPS) and accordingly the corresponding receptor was termed NPS receptor (NPSR) (Xu et al., Neuron 43: 487-497, 2004). The receptor is expressed in brain structures associated with emotional processing, such as the amygdala, the thalamocortical limbic system and the hypothalamus. The ligand is expressed in brain stem structures involved in arousal and stress processing. NPS was found to produce profound arousal, measured as stimulus-independent hyperlocomotion. In collaboration with colleagues at The Scripps Research Institute we showed that NPS could suppress sleep and induce wakefulness. We also found that central administration of NPS produced anxiolytic-like effects, i.e. reduced signs of anxiety, in four different tests of anxiety behavior (Xu et al., 2004). We will continue to investigate further physiological functions of NPS with respect to motor coordination, arousal and attention, learning and memory, as well as possible functions in models of psychotic disorders. For this purpose, we will use synthetic NPSR antagonists and agonists. These experimental agents will be supplied by Dr. [redacted]. of [redacted] ([redacted]).
Committee Discussion: Edits and clarifications need to be made in Click. A scientific justification needs to be given for CO2 euthanasia; the committee recommends deep anesthesia followed by secondary euthanasia. The LAF vet techs do not need to be listed on the protocol. A procedural timing flow chart must be added to "Beta-Arrestin Pathway Testing"All mice that undergo ICV injections and fear conditioning should be category D, and animals that undergo a forced swim test or restraint stress should be category E. Any animal undergoing genotyping should be a pain category C. The experiments "NPSR Agonist Testing – mice" and "NPSR Agonist Testing" need a more detailed account of the requested number of animals. The experiment "NPSR Agonist Testing – mice" needs a more detailed timeline. The experiment "NPSR-CRE Testing" and "NPS-CRE testing" need a substance administration for ketamine and xylazine, the administration procedure for AAV, and a tail snipping procedure. The committee recommends swabbing the injection site to provide a small degree of disinfection for the procedure "Free-Hand ICV-mice ."The procedure "Drugs for Assay Validation and Training" and "Drugs for Assay Validation and Training – rat" needs clarifications to how psychotic episodes induced by drugs will be exhibited. The procedure "Forced Swim Test-mice" needs clarifications to monitoring procedures. The procedure "Drugs for Perfusions-mice" needs edits to pentobarbital dosage. The survival surgery "Dual Cannulation and Jugular-mice 2111216" needs to be updated to reflect new standard operating procedures. The analgesia administration needs clarification, and the committee recommends using septocaine, bupivacaine, or ropivacaine. The procedure "Drugs for Pharmacokinetic Studies-mice" needs edits to the pentobarbital dosage; more details are needed for the intranasal administration and administration of novel compounds. The procedure "Drugs for Perfusion-rat" needs edits to the pentobarbital dosage. The procedure "Dual Cannulation and Jugular-rats 211216" needs to be updated to reflect new standard operation procedures, the analgesia administration needs clarifications, the ketamine/xylazine dosage needs to be corrected, clarifications needs to be

made to Baytril dosage, and the committee recommends using septocaine and bupivacaine or ropivacaine. The procedure “Drugs for Euthanasia” and “Overdose Euthanasia – mice” needs clarifications to pentobarbital dosage. The surgery “Stereotaxic Infusions of Biological Agents-mice 211216” needs to be updated to reflect new standard operating procedures, carprofen dosage needs to be clarified, and the committee recommends that wound clips be removed within 7-10 days. The alternative section needs to be additional searches.
The housing section needs clarifications.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

12. TR202200003 (PROTO201800131)

Protocol Summary: Taken from the PI’s non-scientific summary: Military personnel is at increased risk of experiencing acoustic trauma as a result of hazardous occupational noise exposures such as high-intensity impulse noise, long-term exposure to repetitive impulses, long-term exposure to continuous noise, or exposure to a combination of impulses and continuous noise from various sources such as explosive detonations, gunfire (small and heavy), plane launches, tank operation, shipboard deployments, etc. Thus, the translational goals of the current project using the chinchilla as an animal model of blast-induced auditory injury, and to correlate the structural and functional auditory changes, is well-aligned with accomplishing the military’s mission to maintain the operational readiness of military personnel and to improve the quality of life in war veterans. Impairment due to impulsive noise can include peripheral hearing loss, central auditory processing deficits, vestibular impairment, and tinnitus. These deficits are particularly challenging in the Traumatic Brain Injury (TBI) population, as symptoms can be mistaken for posttraumatic stress disorder, mental health issues, and cognitive deficits. In addition, comorbid factors such as attention, cognition, neuronal loss, noise induced hearing loss, etc., can confound assessment, causing misdiagnosis. Furthermore, some auditory impairments, such as sensorineural hearing loss, will continue to progress with age, unlike many other injuries. In the TBI population, significant clinical challenges are the accurate differentiation of auditory and vestibular impairments from multiple, many times overlapping, symptoms and the development of multidisciplinary rehabilitation strategies to improve treatment outcomes and quality of life for these patients.This research protocol investigates mechanism of neurodegeneration in auditory and vestibular system following noise exposure and/or ototoxic agents. However, ototoxic agents such as carbaryl (pesticide) and Manganese (heavy metal) will be added separately to the protocol by an amendment. The overall objective of this research is to improve the quality of life for Veterans with hearing and balance disorders. Our research aims to advance the knowledge of these disorders and to improve best clinic practices for the assessment and rehabilitation of hearing and balance function in Veterans and the community at large.
Committee Discussion: Edits and clarifications need to be made in Click. The use of CO2 needs a scientific justification, and the committee is recommending deep anesthesia followed by a secondary method of euthanasia. The committee requested the PI define "impulsive noise" and how it is relevant to humans suffering TBI in the essential information section. The experiment "Blast induced auditory/vestibular neurodegeneration" needs clarifications to the statement "10 as standby" related to animal usage.
The procedure "Neurophysiological recordings - Invasive" needs edits and clarifications to the size of the bone removed during the craniotomy, chinchilla's withdrawal reflex, incision preparation, and procedure length. The procedure "sodium salicylate" needs the needle size indicated. The procedure "Substance Administration: Transcardial Perfusion/Fixation, ver.

(Team)" needs clarifications to Fatal Plus dosage and how it will be administered. The procedure "Euthanasia-Cervical decapitation" needs edits to reflect that cervical dislocation is not suitable for adult chinchillas, decapitation is the secondary method of euthanasia, and the dosage of fatal plus needs to be edited. The procedure "Behavioral: Startle reflex test, ver. (Team)" needs edits to the procedural description, how chinchillas are adapted to the startle test cages, startle reflex, and "Preyer's Reflex" needs to be defined. The procedure "Anesthesia induction, ver. (Team)" needs clarifications to the use of pharmaceutical-grade drugs. Personnel needs to add their animal research experience. The housing section needs edits, NPG forms need to be added, and clarifications need to be made to the monitoring chart

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

13. **TR202200004 (BCH01062N)**

Protocol Summary: Taken from the PI's non-scientific summary: The objective of the proposed work is to understand how cells perceive the availability of nutrients in the environment, and adjust their metabolism to accommodate it. We focus on metal nutrients because they are essential, but can be toxic, and therefore maintenance of homeostasis must be carefully regulated. We are working with the bacterium Bradyrhizobium japonicum. We are interested in understanding how B. japonicum senses the metals iron and manganese and regulate gene expression to maintain optimal levels in cells. We are interested in proteins that transport metals into and out of cells, and in regulatory proteins that control gene expression. We propose to use rabbits to raise antibodies against proteins of interest as crucial tools to address specific questions. The antibodies will be used to detect proteins in tissues and cells under various conditions or in various stages in development. Immunoblotting and immunohistochemistry, techniques that require antibodies, are sensitive and powerful techniques for detecting the proteins of interest in tissues and within cells. The antibodies are also used to detect regulatory proteins binding to DNA to regulate genes.

Committee Discussion: Edits and clarifications need to be made in Click. The experiment "Antibodies for B. japonicum" needs to state that the lab will follow SOPs for technical procedures. The procedure "Euthanasia Procedure" needs to state where the blood will be removed from, needle size, and clarifications need to be made to secondary euthanasia method. The procedure "Blood collection" needs to indicate needle size, and clarifications need to be made to blood collection drugs.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

14. **TR202200005 (PMY35088N)**

Protocol Summary: Taken from the PI's non-scientific summary: The objective of this renewal application is to breed various animal colonies in order to maintain the investigator's colonies at this university.

Committee Discussion: Edits and clarifications need to be made in Click. A scientific justification is needed for the use of CO2, and the committee is recommending deep anesthesia followed by a secondary method of euthanasia. The experiments "Breeding" and "Pentobarbital (Fatal Plus)" needs edits to the pentobarbital dosage. Any ear notching or tail biopsy is considered a pain category C, and the animal justification section needs edits to reflect pain categories.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

The IACUC Chair adjourned the meeting at 2:50 PM.