University of Washington
June 20th 2024 IACUC Meeting Minutes

Members Present:  AB   GL   JS   MB
                 AP   GW   JT   MK
                 AW   JFI  SF
                 CC

Members Absent:  BE   GS   JPVH   MRB
                 KG   DM

Opening Business
• The Floor was opened for public comment at 2:32 pm.
• The IACUC Chair called the meeting to order at 2:38 pm.

Confirmation of a Quorum and Announcement
• Quorum was confirmed by JS.

IO Presentation:
An introduction of the ACO3Rs Website (3Rs - Animal Care, Outreach, and 3Rs Program at the UW (washington.edu)).

Approval of the IACUC Meeting Minutes
• The IACUC Chair called for the approval of the May meeting minutes.
  Motion was made and seconded: to approve the minutes as written.
  Further Discussion: none
  Vote: Approved with 9 members voting in favor, 0 against and 3 abstentions.

Attending Veterinarian’s Report

I have checked with the leadership at all sites, and I have three incidents to report from the primate center.

Incident #1
On April 25th, 2024 four geriatric pigtail macaques arrived in Seattle from an institution on the east coast. This shipment was initiated by the receiving PI in January 2024. The animals had previously been part of a long term cognitive study at the prior institution with a collaborator of the WaNPRC PI. They were being transferred to WaNPRC to be added to an existing protocol here for continued cognitive studies. After the animals were selected, the health records were reviewed by WaNPRC veterinary staff who agreed they could likely tolerate shipment despite the identification of a number of underlying age-related diseases.

Q6 of the Animal Justification section of the protocol states, the following: “Identify each source of animals for this protocol if not purchased through DCM or WaNPRC”. The institution from which these animals were shipped was not listed as the source of animals on either the receiving PI’s protocol nor on
the colony holding protocol. Further, at least one of the animals did undergo experimental surgery at the sending institution to induce strabismus. The experimental condition is not part of the described experiment (which does include visual behavioral work) for which these animals were intended.

The shipping SOP at WaNPRC specifies requirements for in-transit communication for incoming shipments (including calls from the transport team at specified timepoints). This part of the shipment SOP was also not followed.

On April 29th, while I was in the process of evaluating the shipment process in general and the decision-making process for this particular shipment, we received an anonymous welfare concern related to this shipment questioning the need to transport geriatric animals for scientific purposes. That concern was evaluated in coordination with the shipment evaluation.

Upon discovery of these incidents, incoming shipments were placed on temporary hiatus. The AV has been working with the shipping and logistics team at WaNPRC to re-evaluate both the process for evaluation of health records and protocol review prior to scheduling shipments as well as for enhanced monitoring during shipment.

The shipping SOP is being updated to include a section on veterinary review and approval. A digital “coversheet” has been created that will be linked to each shipment and will highlight the key responsible parties for each step of approval. The shipping and logistics coordinator has been specifically tasked with verifying animal numbers, animal demographics, and protocol approval when needed. Criteria are being established to determine when animals must be on research protocol prior to arrival – specifically that non-naïve animals that are selected and intended for a specific PI must be included on the IACUC-approved protocol with an explanation of prior research use included on that protocol.

In-transit monitoring has been further enhanced to include more frequent communication from the drivers as well as remote monitoring to allow real-time GPS and temperature-monitoring while in transit.

At this time, I am working closely with the shipping and logistics team to allow incoming shipments on a case-by-case basis until confidence in our processes is restored.

This has been reported to OLAW and will be reported to the USDA.

Are there any questions about this incident? Is there any follow-up that the committee would like to request about this incident.

Discussion:
The IACUC discussed what types of communication structure could have kept all better informed of the decisions made. There are detailed SOPs for transport and reassignment of NHPs that were not being fully followed.

The IACUC also discussed what information should be available for evaluation of fitness for reassignment/transport. This appears to be a non-compliance due to the deviation from SOP in transit communication, and the transfer not being approved on the PI’s protocol, rather than a concern that an animal was inappropriately shipped. There is no indication that any inappropriate decisions were made regarding these animals’ fitness for transport, based on medical records provided prior to transport.
It was unclear if the prior surgery that was identified was experimental or clinical. The surgery should have been flagged, but there is no indication that this impacted the welfare of the animals being transferred.

Motion was made and seconded: to send a letter of counsel to the PI
Further Discussion: Clarified that specific concern is not having this transfer approved in their IACUC protocol prior to transport.
Vote: Approved with 12 members voting in favor, 0 against, 0 abstentions.

Incident #2

A 22-year-old intact female pig-tail macaque underwent intracranial injections as outlined on the research protocol on August 2nd, 2023. The surgery involved a midline cranial incision and the creation of two burr hole craniotomies [one craniotomy over both the superior colliculus (SC) and pulvinar (PUL) areas and one over the predorsal bundle (PDB)]. Using MRI data (from an MRI obtained on July 18, 2023), a robotic arm holding the cannula preloaded with the viral vector was positioned above the entry site for the injection.

The Superior colliculus was targeted first, and all injections to the SC were performed uneventfully.

During the entry on the second location of the pulvinar injection the cannula broke against the dura before entering the cortex.

During the first location at the PDB, the software malfunctioned, causing the robotic arm to perform small uninstructed movements. At this time the surgery was suspended. The burr holes were filled with gelfoam. The skin was closed in two layers and the animal was recovered.

During recovery the animal had a grand mal seizure and was given diazepam which stopped further seizure activity. As recovery progressed, it was noted that the animal did not have full function of the left side of the body and veterinary staff prescribed dexamethasone to manage likely cerebral edema. The animal was closely monitored over the next several days, and veterinary staff documented intention tremors of the left arm and foot which improved over 2-3 weeks.

We cannot definitively state the cause of the post-operative seizure in this animal as seizures can be observed for a variety of reasons in otherwise healthy animals. Per discussion with veterinary staff, the occurrence of seizures following this type of procedure is quite rare. In most cases, animals are asymptomatic following this procedure or may display mild weakness on one side of the body that typically resolves in 48-72 hours.

The protocol, as written at the time of this surgery, specified that animals in this short-term study would undergo a single surgery to create up to 12 small burr holes and perform microinjections of AAV or lentiviral vectors.
The lab scheduled a second surgery to finish the injections. Upon OAW review of the request, an OAW reviewer advised that an amendment would be needed to cover the extra surgery. In the communication that lab stated the following: "Due to an equipment failure, the team was unable to finish, so they were hoping to go back into the existing craniotomy sites to complete the series of injections."

On 8/8/2023, an amendment to the protocol was submitted that added the following statement: "We do everything we can to thoroughly prepare for all surgeries and try to anticipate possible complications, sometimes we encounter unexpected events that preclude us from completing everything as expected. If faced with this situation, another surgical or anesthetic event will be scheduled as soon as possible. Decisions to abort and reschedule a surgery will be made in conversation with the WaNPRC veterinarian(s). Examples of such events include, but are not limited to; computer, surgical hardware, or other equipment failures and damage to sensitive equipment during transportation to and from the Allen Institute for gas sterilization."

On the amendment summary page, Q3 (Describe the rationale for this amendment) was answered as follows: Because we use the brainsight robot for our surgeries events such as software failures (computer crashes) or hardware failures (robot crash) may occur making it impossible to complete all surgical objectives.

This was approved by the IACUC on 8/21/2023

On August 22nd, 2023, the animal was considered returned to normal by veterinary staff and was cleared to complete the remainder of the injections through the existing craniotomies.

On August 23rd, 2023, the intracranial injections into the predorsal bundle and pulvinar areas were completed with no reported malfunctions of the robot. Unfortunately, the animal did not recover from anesthesia despite significant veterinary intervention. The animal was determined to be brain dead and euthanized on the table and submitted for necropsy.

Gross necropsy revealed an approximately 3cm diameter blood clot at the brain stem, oriented to the right side, and hemorrhage along the base of the brain with congested ventral cerebral vessels and submeningeal hemorrhage. The location of the hemorrhage suggested that it was likely secondary to injections.

The protocol states the following in regard to intracranial injections: “No deficits are expected but there are risks associated with neurosurgical procedures that include infection and/or bleeding. Our methods minimize these risks.”

This has been reported to OLAW and will be reported to the USDA.

Are there any questions about this incident? Is there any follow-up that the committee would like to request about this incident?

Discussion:

Members of the committee wanted further information as to why this event was not reported in a timely manner.
The IACUC wanted to know if there was any imaging done between procedures to identify what caused the seizures. Such imaging is possible but not a common occurrence due to the practiced nature of this procedure. This type of imaging is not always a first choice as the state of the animal, including cranial implants, can negatively impact imagining.

Vet staff made decisions based on the clinical disposition of the animal. According to the necropsy findings, we do not know when this clot developed. The surgery malfunction should have been clearly documented in the amendment. The experience was not fully covered in the description/explanation given at the time of the amendment.

What is the history of this type of event occurring in their NHP neuro protocols? Neurological signs in the aftermath of intercranial injections, development of neurological signs are uncommon and resolve in 2-3 days. More severe reactions are rare.

How was this incident not covered in the process of an amendment to continue with surgeries as a “repair or completion”? OAW was informed of the equipment failure, but not that this included any adverse event/clinical impact on the animal afterwards.

Triennial reviews are the format that asks if any adverse events occurred that were unexpected. The amendment process includes the “describe the rational of this amendment” - but adverse events are not directly asked for.

Requested action to update the protocol to include the unexpected adverse events, along with a push for a letter to those overseeing, as this event was not reported to the AV.

The suggestion is to have letters asking the PI and veterinarians who were involved to explain the circumstances of how this has occurred. And to ask the involved individuals about their understanding of the reporting chain and where there might have been a deviation.

**Motion was made and seconded**: to send a letter to the PI and the veterinarians involved

**Further Discussion**: To gather more information on this incident, and to receive an explanation as to why the chain of reporting was not followed.

**Vote**: Approved with 12 members voting in favor, 0 against, 0 abstentions.

**Incident #3**

In November of 2022, it was reported to the IACUC that an animal on 4531-04 unintentionally had water withheld for >24 hours. As a reminder, this animal did not receive the assigned water ration on two consecutive days and then received water late on the third day. The animal did receive enrichment, including produce on at least 2 days. A number of changes were made to the process for providing and documenting water at that time, including the use of a tablet system that automatically sends emails to staff when water is provided.

Regarding that specific incident, at the time it occurred and was reported, the animal was apparently healthy with no lasting impact due to the lack of water. Unfortunately, this animal has developed some clinical issues that may be secondary to this incident. For most of the following year (2023), the animal
tolerated water regulation as outlined in the approved protocol without apparent complications. However, in March 2024, initiation of water regulation at a lower level resulted in acute inappetance prompting veterinary evaluation. Bloodwork indicated inflammation and elevations in some of the renal parameters. Urinalysis indicated isothermuria (meaning the urine was dilute). Ultrasound examination revealed significant abnormalities in renal architecture and enlargement of the left kidney. The animal was treated for presumptive pyelonephritis which improved the clinical signs, but the renal cysts persisted. The veterinarian ultimately diagnosed interstitial nephritis exacerbated by water regulation. The animal remains asymptomatic when on ad lib water, and the veterinarian has mandated that the animal remain on ad lib water for life.

We cannot definitively say that withholding water in 2022 directly caused this animal’s chronic kidney pathology. However, in my experience, spontaneous development of renal disease in young adult macaques is exceptionally rare and thus I felt obligated to inform the committee about this occurrence.

Are there any questions about this incident? Is there any follow-up that the committee would like to request about this incident?

Discussion: none

Protocol Monitoring:

There are currently 26 protocols on enhanced veterinary monitoring. Seventeen of these 26 were placed on monitoring proactively, and the remaining 9 were placed on monitoring as the result of an unexpected outcome. A total of 10 protocols are currently performing the procedure for which they are on monitoring. All PIs on monitoring continue to work with their veterinary monitor.

CLATR Update: New online CLATR training on the lock style and lock position for NHP and vivarium spaces. Training will be rolled out within the next month. Also, a new survey has been generated for training, to provide how well your training has supported IACUC members for their roles. Then the survey will be modified before running to researchers.

OAW Director’s Report – JFI

IACUC Metrics – IACUC metrics are in the meeting folder

Responses to Letters:

RSS – At the May meeting, an incident was reported to the committee in which 5 non-human primates did not receive the required systemic analgesia at the time of either peripheral lymph node biopsy or CSF tap. The IACUC voted to send a letter of reprimand to the manager of RSS. That letter was sent, and a formal response to the letter is still pending but I want to let the committee know that the manager is in contact with OAW and the AV. One update I can share with the committee is that a HoverBoard training session has been scheduled for RSS staff. Following that session, OAW will work with RSS to ensure that RSS leads are added to the associated IACUC protocols so that they can easily access and review the protocol, as well as receive notifications about any amendments. We will update the committee again at the July meeting.
4330-01 – At the May meeting a protocol noncompliance was reported to the committee in which 24 zebrafish transplanted with tumor cells were not monitored as described in the approved protocol. Additionally, a chemotherapeutic agent was administered by an unapproved route, and two groups of fish received a combination of chemotherapeutic agents despite the protocol only being approved to administer a single agent at a time. The IACUC voted to send the PI a letter of reprimand, specifically asking the PI to describe how they will ensure that all lab members understand the importance of protocol compliance, how to access the protocol in Hoverboard, and are aware of any changes made to the protocol. The PI replied to the IACUC’s letter, confirming corrective actions that have already been reported to the committee, and indicating that they have gone over all corrective actions in detail with all personnel in the lab. This included going over the importance of protocol compliance and how to access and review the protocol in HoverBoard. The PI also indicated that they will schedule a HoverBoard session with their IACUC liaison for one of their upcoming lab meetings. I have confirmed with the liaison that they are scheduled to attend a lab meeting with this group in July.

3062-01 – At the May meeting it was reported to the committee that a group has been using approximately 2-6 mice per year to test the potency of a compound prior to proceeding with the experimental cohort. This use of animals for testing was not approved on the protocol. Taking into consideration that this lab had a protocol noncompliance approximately 2 years ago that involved administering a substance at an unapproved dose, the IACUC voted to send the PI a letter of reprimand asking the PI to describe corrective measures that are being implemented to prevent a recurrence. In their response, the PI explained that "This problem stemmed from my misunderstanding that we could use mice that would otherwise be euthanized (because they were progeny generated for an experiment that had the ‘wrong’ genotype) for other purposes (e.g., testing antibodies on fixed tissue).” The PI went on to explain "I now understand that the protocol needs to account for all mice that undergo “an experiment”, even if it is to test a drug at a dose that is approved and routinely used in the lab." For corrective actions, the PI has discussed this situation with their lab staff and explained that, before embarking on a project, they need to check that every mouse used is accounted for on the protocol, and cross-check with the PI.

4249-01 - At the May meeting, it was reported to the committee that an individual had performed surgery on 5 mice without completing required training. While the individual was supervised for the surgery, they had not completed Surgery Laboratory 2, which is required prior to performing surgery even under direct supervision. It was also noted to the committee that this group had had a similar incident in the past. Due to the recurring nature of the incident, the IACUC voted to send a letter of reprimand to the PI, specifically asking them to describe corrective measures being implemented to prevent a recurrence, including oversight of training certification. The PI explained in their responses that in the past they have requested verbal affirmation from trainees that they completed the required training, which this individual provided. However, following this incident it became apparent that this individual misunderstood the requirements and thought the request was about just the online training and not the hands-on portion. To rectify this in the future, all trainees will be asked to provide written documentation of completion of required training. I also followed up with this group’s OAW liaison, and they will reach out to this PI to make sure they know how to review training records in HoverBoard, in case that’s helpful for them.
4481-02 - At the May meeting a protocol noncompliance was reported to the committee in which mice were not given acetaminophen in their drinking water as described in the IACUC protocol. The IACUC voted to send the PI a letter of counsel. The PI responded to the letter and confirmed the corrective actions that have already been reported to the committee, which include reminding all lab members that analgesia is a part of all experiments, reminding lab members that they need to review the protocol prior to starting any new experiments, and a plan to incorporate ongoing reminders about the importance of protocol compliance at future lab meetings.

Noncompliances:
4298-01 – On April 15 2024 it was identified that a research group had been applying a substance called R848 topically on the ears of mice 3 times a week for the previous 4 weeks. There were a total of 16 mice enrolled in this study. At the time the group was approved to administer R848, an immunostimulant, by IP injection daily for up to 7 days, but they were not approved to administer the agent topically and they were not approved to administer it for 4 weeks. Additionally, blood was being collected once each week by retro-orbital collection, alternating eyes. The protocol was approved for a single retro-orbital collection in this experiment, but not for repeated retro-orbital blood collection. An amendment to the protocol had been submitted on March 18th to add this work and it was in process when the work was conducted, but it was not yet approved. This was discovered by vet services while they were following up on a report of eye inflammation in one of the involved cages. The cause of the eye inflammation could not be conclusively determined, and the group promptly initiated treatment as directed by the veterinarian.

The PI identified the cause of the noncompliance as a miscommunication within the lab, with the graduate student mistakenly believing that they were OK to start the study even though the amendment was not yet approved by the IACUC. The experiment was halted immediately once the noncompliance was identified, and the PI has reminded their staff and all trainees to review the active protocol to be sure that all experimental procedures are approved.

As far as previous incidents, there have been none in the past several years. There were two adverse events reported in 2019, both related to dosing errors. The IACUC sent a letter of acknowledgement in response to the 2nd reported adverse event in 2019.

This has been reported to OLAW.

Discussion:
The IACUC felt confident in the steps already taken by this group. The IACUC still wishes to have more direct documentation to ensure this type of miscommunication does not occur again, including a check list to support protocol compliance.

Motion was made and seconded: to send a letter of counsel to the PI
Further Discussion: none
4465-02 – In March 2024, a pilot study was initiated that involved pair-housing two different strains of mice. One of the strains, FVB, was not approved on the protocol. This is a white mouse which is known to be particularly aggressive, although the PI was not aware of this at the time. Five cages were set up, with each cage consisting of 1 male FVB mouse and 1 male C57Bl6 mouse. On March 26th, vet services received a report of fighting in one of the cages, and one mouse had to be euthanized due to severe fight wounds. On April 27th, another report of fight wounds was received, and on the following day, April 28th, a third report of fight wounds was received. Both of these resulted in euthanasia of one mouse in each cage due to severity of the wounds. Additionally, on April 28th there were 2 dead animal reports received for the remaining two cages. In each one of those cages one animal was found dead with wounds, but it could not be determined if the wounds were from fights or received post-mortem. Due to these outcomes, the group halted this pilot.

To provide some context, the intent of this study is for animals to be pair-housed with social partners that will ultimately be used in social behavioral testing where pairs will lever press for interactions with each other. For this test it is important that the animals know each other. Coat color matters due to the machine learning techniques that are employed for video analysis. The software can track the animals exceptionally well if they have different coat colors. If animals have the same coat color, researchers must manually review the videos and correct for any “ID swapping” that occurs, which adds an enormous amount of time and error-potential to the analysis. The rationale for using FVB mice was that these animals have been used in social behavioral tests, and they also have high breeding productivity. This group has previously cohoused c57bl6j with c57 albino mice with success, but were seeking another option for a white mouse due to poor breeding outcomes with the c57 albinos.

The OAW Liaison has met with the PI to discuss this incident and is working with them on an amendment to the protocol to clarify that unfamiliar mice may be cohoused and that c57bl6j and c57 albino pairs do not have a history of fighting. The group is currently working to identify a suitable white mouse.

This has been reported to OLAW.

I found no record of previous noncompliances or adverse events related to this PI.

Discussion: Was the anticipated issue with housing different strains of mice after weaning (including pair housing male mice together of different strains)? This was a pilot study, and the specific strain was not listed on the protocol (which caused the non-compliance). Specifically, one strain of mice is known to be aggressive, something a reviewer would have been able to note and question. The corrective action taken and the receptiveness of the research group is noted.

Suggestion of development of SOP for pair housing multiple strains of mice.

Motion was made and seconded: to send a letter of counsel to the PI.
Vote: Approved with 12 members voting in favor, 0 against, 0 abstentions.

**Standard Procedures/Policies/Guidelines - AS**

2 blood collection procedures in primates with no changes

Motion was made and seconded: to approve the procedures as written.

Further Discussion: none

Vote: Approved with 12 members voting in favor, 0 against, 0 abstentions.

**Semi-Annual Program Review:**

For the semi-annual review of the animal care and use program, the IACUC was split up into groups and each group evaluated different aspects of the program as categorized in a modified OLAW program review checklist. Now we will hear summaries from each of the groups:

- Sections 1, 2, 11, and 12, which include the Animal Care and Use Program, Disaster Planning and Emergency Preparedness, Personnel Security, and Investigating & Reporting Animal Welfare Concerns:
- Sections 3, 5, 6, and 7, which include the IACUC, IACUC Membership and Functions, IACUC Training, and IACUC Records & Reporting Requirements:
- Section 4, IACUC Protocol Review: Sections 8 Veterinary Care and the separate Veterinary Care portions of the checklist: Sections 9 and 10, which include Personnel Qualifications & Training and Occupational Health & Safety of Personnel:

Thank you to each group for your thoughtful reviews. These summaries will be incorporated into the draft semi-annual report to be reviewed at the July meeting.

**Site Visit Survey**

Some of you may recall that in January of this year we conducted a survey of IACUC Members and Alternates focused around IACUC site visits. There’s a detailed summary of results in the meeting documents if you are interested, but I’ll provide a very high-level summary. I want to thank everybody who responded to the survey – the results are very informative and will continue to inform future process improvements.

A majority of respondents expressed that they enjoy doing facility inspections, which was nice to see, and a majority expressed that they felt adequately trained to perform facility inspections. The biggest obstacle for people is not surprisingly time availability and workload, with two-thirds of respondents stating that as a challenge. Several people expressed greater comfort in performing certain types of inspections, for example lab spaces or vivariums, or similarly expressed less confidence in performing inspections of specialized spaces like imaging spaces. There were a lot of really good suggestions for improving training or preparedness for inspections, with many respondents mentioning checklists of some form. One change that
we have already instituted is that our existing site visit checklist, which is shared on our public-facing site for researchers to access, will be attached to the outlook invites for easy reference. That checklist is for reference only, there is no requirement at this time to use it or complete it, but some may find it helpful as an aid. OAW will continue to consider other references or checklists that may be helpful for IACUC members.

A majority of respondents also felt that the current scheduling strategy was effective, although many people had constructive feedback or suggestions for improvement. We are still and will continue to review the suggestions as OAW evaluates the scheduling strategy. There was no apparent consensus on how to improve scheduling, with wide variability among respondents in terms of whether they preferred shorter inspections, longer inspections, inspections earlier in the day, etc.

So again, if you’re interested, I encourage you to look at the detailed summary in the meeting documents, and please let me know if you have any questions or additional feedback. Thank you again.

**Closing Business:**
The Meeting was brought to a close at 4:40 pm.