

University of Washington
June 16, 2016 IACUC Meeting Minutes

Members Present:	AB	JM	MG
	AS	JPVH	ML
	CG (Scientist)	JS	SH
	CH	JPI	SL
	CJ	KL	TB
	JB	*MB	

Members Absent:	CG	MB	NK
	JM	MT	PB

*MB alternate for JPVH

Opening Business:

The IACUC Chair called the meeting to order at 2:32pm.

The IACUC Chair welcomed two new public members to the IACUC.

Approval of the IACUC Meeting Minutes

The IACUC Chair called for the approval of the May 19, 2016 IACUC meeting minutes.

Motion: A motion to approve the minutes as written was made and seconded.

Discussion: None

Vote on the Motion: The meeting minutes were approved with 12 members voting in favor and three abstentions. JB was not present for the vote.

The Benefits of Research – JS

The Benefit Story this month is on the UW community response to combat the emerging threat of Zika virus, which is spread by mosquitoes and causes birth defects, including babies born with abnormally small heads and brains, referred to as microcephaly, often leading to severe lifelong disabilities. Over a dozen groups here at UW are taking a broad variety of approaches to improve testing, treatment and prevention of this devastating disease. <http://globalhealth.washington.edu/news/2016/05/20/what-were-doing-combat-zika-virus>. As with the previous response to the Ebola crisis, we are very proud of our animal researchers who have enlisted in this global fight against a rapidly spreading threat to human health.

Attending Veterinarian's Report

Facility issues, Humidity, Temperature and Lights: None

Protocol Monitoring: There are 15 active protocols on the veterinary monitoring program. Nine involve surgery, anesthesia and surgery, or restraint and surgery. Six involve other procedures including one that involves tumor monitoring and one that involves monitoring of behavioral tests.

The AV suggested removal of the protocol 2153-08, involving behavioral tests. This mouse protocol was added to the veterinary monitoring program at the request of an IACUC member due to the water restriction paradigm on

University of Washington
June 16, 2016 IACUC Meeting Minutes

the protocol. The experiment was performed and the animals acclimated well to the water restriction. However, this experiment will not be repeated and it is being removed from the protocol (3 year removal). The Committee had no objections to removing the protocol from veterinary monitoring.

Adverse events:

- Protocol 2050-01: This protocol is designed to help us understand the molecular basis by which photoreceptors in the retina respond to changes in illumination in order to understand the metabolic components of retinal disease. On May 6, 2016, one black male mouse born April 10, 2016 and weaned May 1st or 2nd was found dead outside of a cage in the animal room. The mouse was ear-tagged and when the group was contacted they reported that a mouse had escaped during weaning that week. They had not notified Veterinary Services or Animal Husbandry when the animal escaped, which is the required reporting process so that steps can be taken to safety re-capture and re-house affected animals. Veterinary Services met with the group and provided re-training on the reporting process. The carcass was very autolyzed and thus the cause of death could not be determined at necropsy. This event was reported to OLAW. No further action was taken by the IACUC.
- Protocol 4261-01: This protocol focuses on understanding signaling proteins which act as regulators of hypertrophy and heart failure utilizing mouse models. On the afternoon of April 29, 2016, 6 mice underwent a transverse aortic constriction surgery. Each received a single dose of buprenorphine HCl approximately 30 minutes prior to surgery. They should have then received additional doses every 8-12 hours for 48 hours, however, they did not receive their next dose until approximately 20 hours later, and the third dose approximately 20 hours after the second. The Attending Veterinarian met with the PI and the group members involved. The individual has been thoroughly retrained and now understands the importance of giving the analgesics as written in the protocol. This has been reported to OLAW.

Motion: A motion to send a letter of counsel to the PI was made and seconded. The letter should include a reminder that all animal work must be done in accordance with his approved IACUC protocol. The Committee requested that the PI reply to the letter indicating the steps that will be taken to ensure that future surgeries and analgesia administration will be given according to the approved protocol.

Discussion: The Committee discussed the training of the individual who made the mistake. The individual has attended the surgery classes and is still under the supervision of a member of the lab. This was the first time that this person had been responsible for post-operative care, and while he should have understood, he just didn't appear to understand how important following the analgesic regimen. TB explained that he was honest and straight forward about the events with her and the veterinary staff.

Vote on the Motion: The motion passed unanimously with 16 members voting in favor.

The Committee discussed possible solutions for improving the challenges of communication when English is a second language.

- Protocol 2384-08: The purpose of this protocol is to study mechanisms and potential treatments for heavy metal toxicity. On April 29, 2016, twelve 24-month old male mice were injected with cadmium chloride IP. The mice were monitored for 6 hours following injection and no evidence of acute toxicity was noted. The next morning, 4 of the 12 mice were found dead in their cage. At the same time, 4 of the remaining mice were noted to be lethargic so the group elected to euthanize the entire cohort and collect tissues. Cadmium testing of the dosing solution revealed that mice were dosed with approximately 8 mg/kg, rather than 2mg/kg, due to a dilution error. Moving forward the group now have multiple lab members check all dosing calculations and a calculation sheet is posted on the hood for immediate reference during cadmium solution preparation. Since this event, the group has performed at least two subsequent experiments with cadmium treatment at 2 mg/kg

University of Washington
June 16, 2016 IACUC Meeting Minutes

with no adverse outcomes. This event was reported to OLAW. No action was taken by the IACUC in response to this event.

HBA Committee:

- Discussed electrosedation used in several fish protocols. The Committee felt that in the context used in these protocols this technique did not fall in the Category E range.
- Discussed clinical cases.

JS reminded the IACUC about the function of the Committee and encouraged other IACUC members to attend.

OAW Director's Report – Summary slide in meeting folder

CTC Review Reminder - Standard procedure for CTC review; When IACUC members review CTC items in HoverBoard, JS reminded IACUC members to leave a note that they have done so.

STI reminded IACUC members to sign up for site visits which start again in July.

DMR Assignment Process – GL summarized the assignment process and asked that people let him know if they are not able to complete their assignments or if they need more time.

The Committee discussed the notification process for HoverBoard. The CTC would still be sent on a weekly basis and reviewers would be notified through the system when they are assigned as DMR. The HoverBoard generated agenda will be sent to the Committee and may be attached to an email. The Committee was encouraged to continue to provide feedback on this process.

Protocol Review

- Dr. Jiang, 4203-03, V7 “Nanogel Carrier for Dual Functional Butyrylcholinesterase: Protection against Nerve Agents and Tunable Nanogel Degradation”

JFI presented the Significant Change to the Committee.

This significant change is adding three experiments in Sprague-Dawley rats to assess the efficacy of a long-circulating bioscavenger to treat or prevent organophosphate poisoning. This significant change is being reviewed by the full committee because it involves toxin administration and spontaneous mortality as an endpoint.

Background: The goal of this group's research is to develop novel bioscavengers against organophosphate toxins. As the group explains in Section A, organophosphates are highly toxic compounds often used as pesticides, and are one of the most common causes of poisoning worldwide, responsible for over 200,000 deaths per year. Additionally, many potent nerve agents developed as chemical weapons are organophosphates. Current antidotes such as atropine can prevent lethality, but do not prevent the severe potential consequences such as convulsions, performance deficits, and permanent brain damage. The group views bioscavenger enzymes as a promising prophylactic and therapeutic approach to protect humans (and potentially animals) from organophosphate poisoning.

Summary of this significant change: This protocol is already approved for non-challenge studies to assess novel bioscavengers, and they are now requesting to add 3 efficacy experiments in Sprague-Dawley rats. These experiments all involve administration of the organophosphate toxin Paraoxon at a dose of 2 x LD50.

University of Washington
June 16, 2016 IACUC Meeting Minutes

Each experiment involves 4 groups of rats – A treated group that receives the novel conjugated bioscavenger (n=6), a group that receives the free unconjugated bioscavenger (n=6), a placebo control (n=3), and an untreated control (n=3). I think the number of animals per group is well justified, with efforts made to reduce animal number, including using smaller numbers in the control groups where 100% mortality is expected.

Experiment 1 is to assess detoxification efficacy. The toxin is administered, followed 5 minutes later by the bioscavenger, and surviving rats are euthanized 24 hours later.

Experiment 2 is to assess prophylactic efficacy of a single treatment. Rats receive the bioscavenger first, followed 3 hours later by the toxin, and toxin administration is repeated every 24 hours for up to 4 weeks.

Experiment 3 is to assess prophylactic efficacy after multiple treatments. Rats receive two bioscavenger injections 2 weeks apart, with the 2nd injection followed 3 hours later by toxin administration, and toxin administration repeated every 24 hours for up to 4 weeks.

Comments about organophosphate toxicity: Organophosphates cause acute toxicity. At this dose death is anticipated to occur within the first 2 hours, and likely early in that time frame. Animals that survive past this initial period are anticipated to recover in the subsequent hours. This toxin acts by inhibiting acetylcholinesterase in the peripheral nervous system, which is essential to nerve function. Expected signs of organophosphate poisoning include salivation, local muscle fasciculation, respiratory depression, and central convulsions or seizures. The primary cause of death is respiratory depression.

In Section A, the group has a good discussion of what factors were considered when trying to reduce potential harm. This includes consideration of surrogate endpoints, clinical signs, symptomatic treatments, and anesthesia. The group concluded that these measures would not be feasible or would impact the outcome of their study.

During pre-review, TB and JS and I discussed potential ways to refine these experiments, including the addition of euthanasia criteria, supportive care, and a more defined and robust monitoring plan. The group was very receptive to our suggestions, and willingly incorporated our suggestions into their experimental design. This included addition of prolonged seizure and weight loss as euthanasia criteria, addition of fluid support for rats in Experiments 2 and 3, and further description of their monitoring plan. I was very impressed by the willingness of the group to consider and incorporate our suggestions.

On a final note, while considering the pain and distress associated with these and other studies, I think it's important to try and remove our personal response to how these animals look when they are experiencing toxicity, and try to objectively evaluate what the animals are likely feeling. I would also like to reiterate the goals of these studies: to develop an effective prophylactic and therapeutic treatment for organophosphate poisoning, which could benefit both humans and animals that are exposed to this common and deadly toxin.

Discussion: The Committee had a detailed discussion regarding the experimental groups, the rationale for the dose selection, the toxicity of the compound, how it's metabolized and the side effects of the compound and monitoring and treatment of distress. The Committee felt that if the experiments went in order, as planned, adjustments could be made to subsequent groups. It was clarified that dosing would be via tail vein.

Motion: A motion was made and seconded to approve the Significant Change with the clarification of the IV route.

Continued Discussion: The Committee continued to discuss the experiment and concerns related to animal distress. The Committee requested that the protocol liaison reach out to the group and ask that they provide feedback to the IACUC at the conclusion of each stage of the experiment.

University of Washington
June 16, 2016 IACUC Meeting Minutes

Vote on the Motion: The Significant Change was approved unanimously with 15 members voting in favor. CJ was not present for the vote. MB served as an alternate for JPVH.

- Faucett, 4209-01, “Collections-based Research and Education at the Burke Museum”
EC presented the 3-year renewal of the protocol to the Committee.

This protocol involves field expeditions to domestic and international destinations to collect specimens for research on non-model organisms (i.e., birds, mammals, reptiles, amphibians and fishes). These research activities include a wide assortment of evolutionary and ecological studies, including morphology, phylogenetics, population genetics, taxonomy, biogeography, phylogeography, biomechanics, ecology and behavior. These activities are conducted by Burke Museum researchers (e.g., curators, collections managers, postdoctoral researchers, graduate students, undergraduates). All research materials are made available to the broader scientific community. Field expeditions often target focal groups of species, but in many cases Museum researchers are tasked with documenting the biodiversity of an entire region and building a collection of specimens for future scientific work. The biodiversity inventory studies are critically important, since they often result in the discovery of new species.

Museum collections can be considered libraries. They consist of volumes, in this case specimens, which illuminate those who use them. Specimens document research. They are the basis for our understanding of the evolutionary relationships between organisms, provide a historical record for comparison with the present, and constitute a reference sample which can be used repeatedly for centuries by scientists, students, law enforcement personnel, artists and resource managers. The specimens in our collections are used by scientists from around the world, as well as many students and professionals in the Pacific Northwest. Those using the collections cannot procure the samples they need any other way, just as those who use libraries could not by themselves procure historical volumes had they not been archived in libraries. All capture/trapping, euthanasia and tissue collection methods are performed in the most humane way possible to avoid unnecessary pain and distress.

Reason for Full Committee Review:

The euthanasia methods outlined in the protocol are all classified as “acceptable” or “conditionally acceptable” by the American Veterinary Medical Association (AVMA), with the exception of thoracic compression (also called rapid cardiac compression) of small birds. This method was classified by the AVMA as “conditionally acceptable” until 2013, when the updated AVMA Guidelines on Euthanasia revised the classification to “unacceptable.”

In response, the Ornithological Council put out a position statement on the reclassification, concluding: “Thoracic compression is humane and often the most humane alternative available for certain kinds of research and in certain situations. Notwithstanding the AVMA’s unfortunate and unsupported determination, its use should not be limited when it is the most humane alternative available (as detailed in the accompanying fact sheet) or the most practical among available humane methods.”

There has also been speculation that the AVMA will once again revise its classification of this method, most likely back to “conditionally acceptable.” However, since the guidelines have not been updated, inclusion of thoracic compression of small birds means this three-year renewal must be reviewed by the IACUC today.

Rapid Cardiac Compression/Thoracic Compression (taken from the Ornithological Council Fact Sheet on Thoracic Compression).

The euthanasia method is widely used for collection of ornithological specimens and tissue samples. It involves holding the bird between the thumb and forefinger of one hand. The forefinger of the other hand is placed against the ventral edge of the sternum. Squeezing the fingers together rapidly with the force of a hard

University of Washington
June 16, 2016 IACUC Meeting Minutes

pinch in the space above the coracoid prevents air from entering the air sacs and causes the heart to stop. The need for an intact specimen, including an undamaged skeleton, precludes the use of force that would be sufficient to break the sternum or ribs. The bird loses consciousness within a few second, and continued pressure on the thorax ensures that the heart will not restart. Death follows quickly thereafter.

Protocol 4209-01 provides the following additional information:

“Only desirable specimens of an appropriate size are euthanized via Rapid cardiac compression/thoracic compression. This technique is only used on species weighing < 200g. Not used on diving birds. Used only when shotgun is not an option. Also used on specimens (of acceptable size) not euthanized by initial gunshot.

The birds euthanized under this protocol are being archived for use in future research. Research that has yet to be defined. We cannot afford to contaminate these specimens risking making them useless in the future. Thoracic decompression gives 'very fast' loss of consciousness in birds (less than 5 seconds for small birds).”

Motion: A motion was made and seconded to approve the protocol as written.

Discussion: The Committee discussed the rationale for the procedure and that it must be done correctly and on the right size of bird. The PI explained that only the ornithology group would be performing the procedure and he summarized the training that is required of these individuals. It was stressed that specimens are being collected in the field and additional equipment required for anesthesia could impact the risk of injury to humans transporting the equipment. Additionally, all specimens are being archived for long-term reference, and they don't know what the material will be used for in the future, which could be hundreds of years down the road.

The Committee discussed pain or distress associated with the procedure, and it was generally thought to be more distressful than painful, however, a definitive answer is not known. As this procedure is a deviation from AVMA euthanasia recommendations, the procedure will be reviewed by the Harm Benefit Analysis Subcommittee for a recommendation on pain/distress classification.

Vote on the Motion: The protocol was approved unanimously with 15 members voting in favor.

Husbandry Limitation Petition – JS

JS summarized the husbandry limitation request for the Committee.

The Chavkin lab has petitioned for Limited Husbandry of their animals. They want to handle all of their animal care themselves, and limit the care provided by DCM staff because they are performing sensitive behavioral tests to measure stress and anxiety. Having unfamiliar DCM personnel enter the room and inspect the animals could be reasonably expected to affect the outcome of these experiments.

After discussion with the group's liaison in OAW, the Chavkin group has indicated that they would be amenable to having a member of the DCM staff enter the room once a week for in-person health checks of the animals while a member of the lab is performing their regularly scheduled cage changes. The Husbandry Limitations Subcommittee has reviewed this request and does not think that this is sufficient, and does not recommend approval of this petition unless daily remote video camera checks by DCM personnel are added to the protocol. Video cameras have been set up to allow remote monitoring of animals belonging to other groups, and appear to be working well. At least so far, UW has been picking up the cost and done the work of setting up the video system.

Motion: A motion was made and seconded to approve the husbandry limitation petition as written. A yes vote would mean that IACUC members are approving the Husbandry Limitation petition as requested, with a single

University of Washington
June 16, 2016 IACUC Meeting Minutes

once-a-week in-person inspection of the animals by DCM staff. A no vote would mean that in keeping with the majority recommendation by the subcommittee, the petition will be denied and the group will continue to work with OAW and DCM to try to incorporate daily video monitoring, in addition to once-weekly, in-person health checks.

Set up of video cameras may take up to 3 months. If it could not be done in that time-frame, the issue should be brought back to the IACUC for a status update.

Discussion: The Committee discussed the behavioral experiments, which are very difficult to centralize and can be influenced by human individuals as well as the importance of providing the very best animal care, as provided by trained professionals as their primary job.

The Committee discussed the use of cameras to monitor the animals remotely. Moving forward, it was suggested that this should be a standard option that can be used by PIs and not require them to petition, a policy could be developed.

Vote on the Motion: The husbandry limitation petition was not approved with 3 members voting in favor and 12 opposed.

IACUC Policy

Frog Oocyte Harvest policy: ML summarized the changes made to the policy. Molly will get me the modified version. It was noted that the AARC revision date that is referenced in the policy should be updated.

Motion: A motion was made and seconded to approve the policy with the suggesting administrative corrections.

Discussion: None

Vote on the Motion: The policy was approved unanimously with 14 members voting in favor. KL was not present for the vote

HoverBoard – EC encouraged members to complete their training and give feedback to the OAW. OAW is moving forward with putting protocols into the system and there is a one currently in review.

Closing Business:

The Meeting was brought to a close at 4:52 pm. The floor was opened to public comment.