

WASHINGTON STATE UNIVERSITY

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Meeting minutes March 31st, 2021

Voting Members: MCt, NW, SM(joined 3:05pm), MB, JB (left 4:15pm), LF, GG, MHk, MJH, SJ, JK, RK (joined 3:45pm), LO (left 4:55pm), GR, ZW, JW (joined 3:10pm), LM (alternate for RK until 3:45pm)

Alternate Members and Staff: AE, CH, KK, VC, MK (alternate), GA (alternate)

Quorum: 16 of 22 voting members present

I. Call to Order

- Meeting called to order at 3:03 pm.

II. Full Committee Review and Approval

- *Minutes of February 2021 meeting*
The minutes of the last meeting were reviewed. A couple of revisions were submitted via email and incorporated into the minutes.

Committee action: By a majority vote of a quorum, the committee approved the minutes as written.

11 Approved, 0 Opposed, 3 Abstained

- ASAF 6895 (*Circadian rhythm disruption exception to The Guide*)
An OCV Veterinarian gave an overview of the protocol. This is a new protocol that will use mice as a model to study the effects of circadian rhythm disruptions on melanoma characteristics. To perform the circadian rhythm studies, the protocol requests a light cycle exception to *The Guide*. Mice will be exposed to chronic jet lag, where repeated eight-hour advances of the dark-light cycle will occur every two days for eleven days. The changes in light cycles will not affect weekly cage changes. Other factors investigated in this study will be the effects of food restriction (8 hours) and physical exercise (up to 30 min of daily, voluntary exercise) on tumor characteristics, and the effects of vitamin C supplementation on regulation of circadian rhythms. The protocol started review under DMR and the PI has answered the reviewers' questions and modified the protocol as needed. There were no other concerns from the committee regarding the protocol or exception to *The Guide*.

Committee action: By a majority vote of a quorum, the committee motioned to approve ASAF 6895.

16 Approved, 0 Opposed, 0 Abstained

- ASAF 6929 (*Circadian rhythm disruption exception to The Guide*)
The AWP Assistant Director gave an overview of the protocol. This is a new metabolomics protocol that will use mice to study the effects of circadian rhythm disruptions on tissues. To perform the circadian rhythm studies, the protocol requests a light cycle exception to *The Guide* where animals will be subjected to one day (24 hours)

of constant light followed by perturbations of dark periods. The committee had no issues with the light cycle exception but agreed that there were still outstanding questions regarding other aspects of the protocol and funding source that needed to be addressed. However, it was determined that this could be done via DMR.

Committee action: By a majority vote of a quorum, the committee motioned to send ASAF 6929 to DMR.

16 Approved, 0 Opposed, 0 Abstained

- ASAF 6924 (*Room temperature exception to The Guide*)
The Attending Veterinarian gave an overview of this protocol. This is a three-year renewal of a protocol that investigates the impact of different diets and dietary bioactive compounds on the microbiota and the effects of cold exposure on stimulation of brown fat metabolism in mice. To perform brown fat metabolism studies, the protocol requests a room temperature exception to *The Guide*. The mice are kept in chambers at 4°C for 7 days in an animal room in the vivarium. To avoid cold exposure-related issues, infant mice are excluded from this study and participant mice are provided with bedding material to allow for thermoregulation. This exception to *The Guide* has been approved in previous versions of this protocol and no animal welfare issues related to temperature have ever been observed. There were no concerns about the exception, but the protocol has outstanding questions that were asked via DMR that need to be addressed before approval.

Committee action: By a majority vote of a quorum, the committee motioned to send ASAF 6924 to DMR.

16 Approved, 0 Opposed, 0 Abstained

- ASAF 6509 (*Addition of category E activities*)
The Attending Veterinarian gave an overview of the project. This is an amendment to an already approved protocol that requests the addition of several procedures, including pain/distress category E activities. New procedures include passive avoidance, elevated maze, and seizure threshold tests. This protocol was pulled for FCR due to the seizure threshold testing, where a mouse strain susceptible to epilepsy will have seizures induced by direct electro-stimulation. When the seizure threshold is reached, the mice will be euthanized. The committee discussed the amendment and asked questions. There were a couple of questions about the duration of the seizure threshold procedure and device used that were answered by the Attending Veterinarian.

The AV and other committee members agreed that several questions have already been addressed but there are still some outstanding issues to be resolved. Outstanding questions include clarifying the duration of the electric stimuli and the creation of a humane endpoint chart. It was determined that this could be done via DMR.

Committee action: By a majority vote of a quorum, the committee motioned to send ASAF 6509 to DMR.

16 Approved, 0 Opposed, 0 Abstained

- **Policy #22: Principal Investigator on IACUC Protocols**
The AWP Assistant Director gave an overview of the Policy, whose purpose is to outline the requirements to be a Principal Investigator (PI) on a WSU IACUC Protocol. It was updated for its three-year review to both update language and add a section about the expectations for PIs that maintain WSU IACUC protocols. There were a couple of questions from the committee that the AWP Assistant Director answered.

Committee action: By a majority vote of a quorum, the committee motioned to approve Policy #22.

16 Approved, 0 Opposed, 0 Abstained

III. Report by the Attending Veterinarian

- The AV presented the following Adverse Event that was received in March:
ASAF 6869: This is a protocol that uses mice for antibody production. In February, twelve mice received the first dose of an immunogenic mixture plus adjuvant followed by two booster immunizations with the antigen mixed in with Freund's Incomplete Adjuvant. Fifteen days after the first immunization, 7 (out of 12) mice developed granulomas at the injection sites that ulcerated. The animals were treated, and the lesions resolved. The first booster immunization contained half the amount of antigen as the first injection, but still 8 (out of 12) mice developed injection site ulcerations 12 to 16 days after the booster. Again, the animals were treated, and the lesions resolved. A second booster injection was given, and the mice were euthanized 7 days post-injection. No ulcerations were observed during the time between the second booster injection and euthanasia.

It was determined that the original immunization with the adjuvant-antigen mix for xx animals mistakenly exceeded the volume described in the protocol by 50 ul, which may have some correlation with the first occurrence of the ulcers. The vet tech who performed the injections has since been retrained and directions for immunizations have been made easily available to prevent future mistakes. After the animals were euthanized at the end of the study, necropsies were performed, and samples of the granuloma sites were cultured for possible infection. Cultures of the antigen/adjuvant mixture revealed no signs of contamination. The PI submitted an amendment to this protocol to update the adverse events section to include the potential for ulcers, treatment options and increase the pain/distress category. There are plans to further investigate the cause of the ulcers, which will be reported to the committee at a future IACUC Meeting.

- The AV presented the list of VVCs approved in March. There were no questions from the committee.
- The AV updated the committee on the following items:

- *ASAF 6686*: This is an update on the necropsy findings of a protocol that uses mice to investigate the anti-tumoral properties of a photodynamic compound that is expected to destroy tumorous cells upon focal exposure to a laser beam. Previously, a group of five mice became lethargic after treatment, and were subsequently euthanized. Necropsy was performed and the histology results revealed extensive infiltration of the tumor into the skeletal muscle surrounding the sciatic nerve, consistent with the hind limb paralysis observed in one animal, in addition to muscle necrosis degeneration caused by the photodynamic compound. More recently, another group of mice was treated with a lower dose of the photodynamic compound and shorter duration of laser exposure and all recovered. They did experience minor lameness that was treated with analgesic drugs.
- *Eastlick Temperature*: The OCV Assistant Director gave an overview of the temperature-related occurrence in the Eastlick building that affected two mice rooms and one rat room. A work order was placed after an abnormal increase in room temperature was reported, where temperatures reached ~87°F. The animals were monitored closely during this event and no adverse effects were observed. Later it was discovered that the pressure regulator was installed incorrectly, causing heat to flow into the animal rooms. The regulator has since been reinstalled and the room temperature went back to normal range.
- *Eastlick Construction*: The construction in the aquatics area at Eastlick continues and it had no cause-effect relationship to the above-mentioned temperature-related issue.
- *Bustad*: The Bustad renovations continue and the facility is not open yet.

IV. IACUC Activities

- Holding protocol: none
- Spring Site Visits
The 2021 Spring Site Visits are currently ongoing.
- Non-compliance
ASAF 6031 (Discussion of post-op analgesia amendment and non-compliance).
This protocol uses mice and rats as experimental models to investigate the biochemical regulation of sleep. It was recently cited for a non-compliance after eleven rats underwent surgery for placement of a jugular vein catheter – a procedure done for training purposes that was not covered by the protocol, and implantation of a thermosensitive transmitter into the abdominal cavity (approved in the protocol). Jugular catheter placement failed in 7 (out of 11) rats and a second surgery was performed on those animals for catheter replacement. The protocol recently underwent a three-year

renewal, where it was asked by the reviewers at that time if jugular catheters would be used. At the time, the PI indicated that they would not be and removed the related reference of them from the protocol. The procedure was never added back to the protocol when the PI did start implanting them. Additional issues noted are as follows:

- Surgery records were not adequately maintained for the jugular catheter surgeries; instead, an outdated, pre-printed surgery log that did not accurately reflect the surgeries performed was used;
- The analgesics used were not consistent with what was approved in the protocol for the procedure (ketoprofen was used instead of carprofen);
- The ketoprofen and buprenorphine used for analgesia were expired.
- The buprenorphine used for analgesia was not properly recorded in the controlled substance log and the incorrect version was used (regular instead of SR).

As this protocol is supported by a NIH award the protocol deviation will be reported to OLAW. The PI indicated that they had previously decided to remove the jugular catheterization procedure to be able to submit the three-year renewal of the protocol before its expiration and had intended to add it back later, but forgot prior to performing the procedure. The PI also provided an explanation for the use of ketoprofen instead of carprofen by affirming that they mistakenly thought they were the same compound. The PI has since agreed to take the following actions:

- Submit an amendment to ASAF 6031 to re-add the jugular catheterization surgery and clearly define the post-op analgesia plans;
- Update all expired supplies within the lab;
- Re-review all relevant IACUC policies;
- Allow for additional PARs and review of surgery templates.

The committee expressed some concern regarding the case and offered suggestions for rectifying the situation. The actions required by the committee are as follows: implementation of a period of one (1) year of oversight of the project by the OCV and AWP, followed by a project reevaluation; the submission for IACUC approval of a protocol amendment to include the new procedure (i.e., jugular vein catheterization); and the retraining of personnel by the OCV. The IACUC agreed that future animal activities under the protocol should be suspended until all corrective actions have been completed.

Committee action: By a majority vote of a quorum, the committee motioned to implement the above-mentioned actions related to the non-compliance and to suspend the animal procedures on this protocol until all stipulations have been met and verified by the AWP Office.

14 Approved, 0 Opposed, 1 Abstained

- There were no new records requests.

V. Post-Approval Review Report

Reporting Period: February 25th, 2021 – March 31st, 2021

The PAR Reviewer gave an update for the month of March. There were two VVCs recommended, including adding a euthanasia room and adjusting the route of analgesia in a protocol.

PAR Totals			
E Class	D Class	C Class	B Class
0	4	3	0

Summary of Findings	
<i>Sx/ Aseptic Technique</i>	0
<i>Surgery/ Anesthesia/ Analgesia/ Medical Records</i>	1
<i>NonSx Proc</i>	0
<i>Drug & Chemicals</i>	1
<i>Personnel/ Training Records</i>	0
<i>Procedure Location</i>	2
<i>Husbandry/ Facility</i>	0
<i>Euthanasia</i>	0
<i>Protocol Amendments/VVC</i>	2
<i>Other</i>	0
<i># of Total Table Tops & Observations</i>	7

VI. Minority Report

VII. Adjournment

- Meeting adjourned at 4:58 PM.
14 Approved, 0 Opposed, 0 Abstained
- Future IACUC meeting dates –
 - 2021 (**Cue 512 or Zoom**): April 28th, May 26th, June 30th, July 28th, August 25th, September 29th

Appendix 1: Protocols Resolved since the last meeting.

# New Protocols	#Amend	#Expired	#Annual Renewals	#VVC	#Adm. Amend
20	17	8	36	8	53

Appendix 2: IACUC Training since the last meeting

Training course	# Participants
<i>Principles of Animal Care (Formerly AAS)</i>	23
<i>Refresher: Principles of Animal Care</i>	8
<i>Occupational Health and Training-Animals</i>	13
<i>Satellite Animal Housing Location Training</i>	4
<i>Aseptic Surgical Technique Online</i>	6
<i>Aseptic Surgical Technique Hands-On</i>	7
<i>Basic Rodent Handling Hands-On (including euthanasia)</i>	6
<i>Rodent Euthanasia</i>	0
<i>Verification of Euthanasia Proficiency</i>	1
<i>IACUC SOP 9, 11, 12</i>	0
<i>Research Specific</i>	3
<i>Anesthesia (USDA-covered species)</i>	0
<i>Tubing Cattle and Sheep</i>	0
<i>Herd Health (species specific)</i>	0

Appendix 3: Protocols currently under review

Currently Under Review	Pending 6+ months
39	0