

University of Washington
May 18, 2017 IACUC Meeting Minutes

Members Present:

AS	*JK	JFI
CG (scientist)	JM	MG
CH	JPVH	SH
JB	JS	TB

Members Absent:

AB	CG	SL
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* JK Alternate for ML

Opening Business

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

Noncompliance Update – WaNPRC Director

JS introduced MM, the WaNPRC Director who was present at the meeting to provide an update on the steps taken in response to the recent noncompliances at the WaNPRC.

MM began by thanking the Committee and commenting to the IACUC that he and members of the WaNPRC unequivocally support the IACUC and the work that the members do and appreciate the value that the IACUC provides to the WaNPRC. MM explained that from his perspective the WaNPRC has a lot of dedicated, experienced people who take seriously their responsibility for the animals in their care and their contribution to excellent science.

MM then discussed the WaNPRC management structure and his management philosophy, which was to operate with an open, collegial, environment, and to support the staff with education opportunities to advance their abilities. MM stressed the importance of work/life balance, which is something that will be stressed to employees moving forward.

MM summarized the actions taken by the WaNPRC in response to the recent noncompliances and explained that in preparation of his written response ([attached](#)) to the IACUC's questions, he interviewed about 20 people, who were behavioral management specialists, veterinary technicians, veterinarians and animal care technicians and asked them to provide answers to the Committee's questions as appropriate.

Corrective action:

- 1) Center wide changes were put into place to retrain staff.
- 2) A change was made to the annual recertification process.
- 3) A buddy system for am and pm room checks was initiated. This change was started at Western but will be implemented in other areas.
- 4) They worked with Human Resources to determine the appropriate action taken for the technician involved with the dehydration incident. MM stressed to the Committee that prior to this issue, the technician was an outstanding employee.
- 5) A system has been implemented so that it is easier to track cage changes in real time.
- 6) During the review of the situation it became clear that excessive overtime may have played a roll and will be monitored moving forward and MM again stressed the importance of work life balance. To address this they will work to redistribute some duties. MM noted that the individual who was

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responsible for the cage change noncompliance is a hardworking, dedicated employee, and overtime may have been an issue in her case.

- 7) To address the anesthesia record noncompliance, the supervisory veterinarian has made changes in the procedure and retrained all the staff to chart these anesthetic events appropriately. In addition, she has assigned two people to each procedure, rather than just one, in order to help provide adequate manpower to do the charting and still properly monitor the anesthesia, especially around the times of transport and repositioning in the MRI.
- 8) They will continue to work to foster an environment of open communication and employee recognition and advancement.

The Committee discussed with MM the possibility of training nonhuman primates to check their own lixits, staffing, and how to improve communication. MM agreed to provide the Committee with updates on the progress of positive changes made by the WaNPRC.

Protocol Review

- **Kiem, 3235-04 “Cell and Gene Therapy for HIV Cure”, Significant Change – JFI**

A full committee review was requested for this amendment due to the increase in the number of laparoscopic surgery procedures. This amendment was discussed by the harm/benefit subcommittee in April, and modifications were made to the amendment based on the pre-review and the subcommittee’s discussion. These modifications included strengthening the scientific justification, clarifying the maximum number of surgeries an animal might have, clarifying the maximum combination of procedures an animal may have at once and how long that would take, and confirming that projects will adhere to the IACUC Policies for blood collection, primate procedures, and laparotomies.

Background: This group’s focus is the development and application of cell and gene therapy strategies to treat HIV infection. There are 3 projects covered on this protocol, each project involving multiple experimental cohorts. Project 1 studies the impact of chimeric antigen receptor T-cells (CAR T-cells) on SHIV infection; Project 2 studies the impact of eCD4-Ig, an engineered antiviral antibody, on SHIV infection; and Project 3 seeks to understand how vaccination and T-cell gene therapy may synergize to combat SHIV infection. There are a total of 148 macaques approved on this protocol.

Amendment: In this amendment, the group is requesting to increase the total number of laparoscopic mesenteric lymph node (MLN) surgeries that an animal may undergo from 3-4 (currently approved) to 5. The group indicates that they’ve consulted with WaNPRC veterinarians, and that the request is based on the fact that lymph nodes are one of, if not the central physiologic site in which HIV “hides” in a latent state during suppressive antiretroviral therapy. Increasing the maximum number of laparoscopic surgeries to 5 will allow additional monitoring of immunological properties prior to antiretroviral therapy and/or SHIV infection in order to construct a more accurate “baseline” prior to infection or intervention.

The protocol is approved for multiple surgical sampling procedures, including upper and lower GI biopsy, and peripheral lymph node biopsy. The approved protocol indicates that at each of the approved surgical time points, optional mesenteric lymph node and/or spleen biopsy may also be performed, with a general statement that “Up to 4 laparoscopic procedures will be performed on any animal.” In this amendment, they increase that number to 5, add a much more thorough justification of the significance

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of MLNs, and describe the 5 phases of the studies in which the samples may be collected. The amendment indicates a minimum of 1 month between laparoscopic surgeries, although from the study timelines the time between laparoscopies would generally be much longer. They also clarify that spleen biopsy is optional during laparoscopic surgery, and they are not requesting an increase in the number of spleen biopsies, which remain at a maximum of 4. They do expand on the 4 study phases in which splenic biopsies may be collected. The protocol retains the language that “Animals will be evaluated by vet staff prior to laparoscopic procedures. If adhesions are present and considered significant, future laparoscopic procedures will not be performed.”

The group also put significant effort into updating the experiment descriptions and the multiple surgery information to clarify the maximum number of surgeries that each cohort of animals may undergo, as well as clarify the maximal combination of procedures that an animal may undergo at each sampling time point. They also clarify how long those procedures are expected to take both individually and when combined into a single anesthetic event. Note, the amendment is not adding or changing any other procedures or altering the timeline of these other procedures or of the studies, but rather providing additional information and clarification.

I want to commend the group for their effort in addressing all of the questions and concerns raised during pre-review and providing the additional information and justification requested to help the IACUC conduct a harm/benefit analysis. I do think this group understands and respects the important role of this committee.

The Committee discussed with the lab member the specifics of the procedure and the plan for adhering to the adhesion policy and for monitoring the welfare of the animal and the adhesions that develop.

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

Discussion: TB commented that the justification has greatly improved and the biopsies are collected over a long period of time so she is not as concerned about animal welfare. She reminded IACUC that each surgery causes pain and distress. Analgesics are given to address this, but it does not totally prevent it. These are not terribly invasive, so the concern of the procedure is less. The Committee continued to discuss significant change, with emphasis on the overall harm/benefit of the procedure, multiple surgical sites, and the development of adhesions, and the importance of making sure that the clinical veterinarians have correct information to assess the animal. CH explained that they won't know if there are adhesions for the most recent biopsy so decision about adhesions and whether or not to move forward for collection would be made during the next surgery.

The Committee discussed the possibility of adhesions and the applicability of the current adhesion policy for this specific method of collection. It was proposed that a policy revision be presented at the next IACUC meeting.

Vote on the Motion: The Significant Change was approved unanimously with 12 members voting in favor.

- Dr. Jiang, 4203-03 “OP Neutralization” - MN

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This Full Committee Review (FCR) includes review of a Progress Report, provided by the group, and an Amendment to their protocol. These items are brought to FCR because of efficacy studies in which rats are administered an organophosphate toxin with the possibility of side effects to include muscle twitching, salivation, tremor, and respiratory depression, and because death is used as an endpoint. There are other non-efficacy studies on this protocol that do not use toxin and they are not part of our FCR.

Background:

The goal of this work is to develop a novel treatment against organophosphate (OP) toxins. The particular treatment the group is working on is a long-circulating bioscavenger enzyme. Bioscavenger enzymes are a promising alternative treatment, as they can neutralize or decompose OP compounds in the bloodstream, before toxicity occurs. Organophosphates are highly potent neurotoxins commonly used as insecticides 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, incapacitation, performance deficits, and permanent brain damage. The group views their biomaterials as a promising prophylactic and therapeutic approach to protect humans (and potentially animals) from organophosphate poisoning.

These studies are all done in Sprague Dawley Rats.

The OP used is paraoxon at a dose of 2 x LD50

The Bioscavengers used are bare and modified organophosphorus hydrolase (OPH).

The first item under review is the Progress Report.

The studies in this report were reviewed and approved at a FCR in June 2016. As part of the committee's approval it was requested that the group provide feedback to the IACUC at the conclusion of each stage of the experiment. The group has completed some experiments and provided a Progress Report.

Progress Report Summary

The goal of these experiments was to evaluate the efficacy of 2 different long-circulating bioscavengers. bare OPH and OPH-nangel, The two areas of investigation are detoxification and prophylaxis.

Efficacy Experiments:

Experiment 1 (detoxification model): OP was injected, followed 5 minutes later by bioscavenger or control, and in the approved experiment if any rats had survived beyond 24 h they would have been euthanized.

Control: 2 groups (saline, nanogel), 3 rats/group

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Bioscavenger: 2 groups (bare OPH, OPH-nanogel), 3 rats/group (6 animals were approved, but only 3 animals were used. Based on results no more rats were used and the experiment was revised)

Results:

Control rats showed serious OP intoxication within 5 min of injection and 100% mortality within 15 min.

Bioscavenger treated rats all behaved normally until euthanasia without any mortality.

These results indicate that both tested bioscavengers protected the rats from poisoning.

Experiment 2 (prophylactic treatment): Bioscavenger was injected first, followed 3 h later by OP. Rats were observed for 2 h. After 24 h, another round of OP administration and observation for 2 hours. This is repeated every 24 h for up to 4 weeks.

Animals were scored based on the following table:

Table 1 Intoxication score definitions

Intoxication score	Symptoms
1	Slight to moderate muscle twitch
2	Muscle twitch; Salivation
3	Salivation; Tremors
4	Serious tremors; Salivation; Respiratory depression

Control: bare OPH can be considered the control

Bioscavenger: 2 groups (bare OPH, OPH nanogel) (6 rats/group)

Results:

3h post bioscavenger injection, all rats survived OP challenge without any observable symptoms. One day later, when challenged by the second OP injection, all six rats in the bare OPH group exhibited serious symptoms and five died within 1 hour. The surviving rat recovered from symptoms within 24 h. By contrast, all rats in the OPH-nanogel group did not show any symptoms post OP challenge. After the third dosage, the last rat in the bare OPH group died within 15 min, while all rats in the OPH-nanogel group were still very well protected. The OP intoxication symptoms appeared after the sixth OP challenge for the OPH-nanogel group and 3 rats died. The 3 remaining rats died after the seventh OP injection. These preliminary results demonstrated the superior protection against OP poisoning offered by the OPH-nanogels.

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Amendment

Submitted to revise efficacy experiments to:

1. Address a request by the funding agency to investigate the bioscavengers administered by the subcutaneous (SC) and intramuscular (IM) route, in addition to the already approved intravenous route (IV), because SC and IM injections are more realistic than IV in the battlefield.
2. Improve the Efficacy Study design based on preliminary results and discussions with experts at the United States Army Medical Research Institute of Chemical Defense (USAMRICD). (e.g., different bioscavenger dosages will be tested and blood collection has been added)
3. There are some changes made to the non-efficacy studies, but all questions about those procedures were answered in pre-review. The changes are to remove the pharmacodynamic study and combine the immunologic study with the pharmacokinetic study. Animal numbers were significantly reduced from 528 to 192.

Summary of revised Efficacy Studies:

These experiments all involve a single administration of the OP toxin at a dose of 2 x LD50, the same dose as already approved. All rats will only receive a once only injection of OP, no repeated challenge. Symptoms will be observed and recorded within 90 min post injection. Some rats are expected to die within minutes (e.g., controls and others not protected by adequate bioscavengers), but the rats that receive native or modified bioscavenger may recover from intoxication. Each test group of rats contains 6 rats, whereas the control groups only have 3. (Fewer control animals is a refinement in the original approved experiment to decrease the number of rats where 100% mortality is expected and is kept in the revised experiments)

Experiment 1 assesses detoxification efficacy – a once only OP administration is administered, followed immediately by bioscavenger (5 doses tested, each in a different group of rats), and surviving rats are euthanized 90 minutes later. Blood is collected before and after OP administration.

Experiment 2 assesses prophylactic efficacy - a single bioscavenger treatment prior to OP administration. Bioscavenger is administered (3 routes IV, SC and IM), followed by OP challenge (4 time points, only one in each rat). Blood is collected before and after OP administration.

Animal numbers have been increased from 54 to 210 to account for the additional groups of rats needed for testing different doses of bioscavenger and different time points for OP challenge, with 63 rats being in pain Category E. (based on the groups preliminary results, they calculated that 30% of animals are expected to have intoxication symptoms or death, while 70% of the animals should be well protected by the bioscavenger.)

Improvements made to decrease potential pain and distress:

1. Rats are euthanized if they survive a maximum of 90 min post toxin injection in both of the efficacy models. There is no prolonged survival.
2. All rats will only receive a once only injection of OP. No repeated challenges of toxin.

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3. There are no saline or nanogel controls in the prophylactic experiment, thus reducing the number of animals used.
4. Control groups only use 3 animals per group as mentioned above, which also reduces the animal numbers used.

These improvements have the potential to greatly reduce both the duration and degree of pain and distress.

The group has a good discussion in HB about the considerations they made to reduce potential harm, and to address using death as an endpoint. In summary,

- death is necessary as an endpoint to mimic the real-world situation where acute toxicity often results in death and the aim is to neutralize the toxic effects of OP, thereby alleviating the worst symptoms of lethal toxin exposure and potentially providing a therapeutic solution
- alleviating symptoms may interfere with experimental results - anesthesia was considered, but the changes in blood flow and the effects on the liver will likely change the model significantly
- the rapidity of death precludes the use of surrogate endpoints - rats will experience acute death with minimal time between morbidity, moribundity and death, therefore, utilizing biomarkers is impossible
- non-death endpoints do not offer the necessary information to assess efficacy
- respiratory distress cannot be used as an endpoint because rats may still recover after showing signs and rats that do not show signs of respiratory distress may still die
- animals with OP toxicity seizure, and animals that seizure longer than one minute will be euthanized – a refinement that was in the original protocol

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

Discussion: The Committee discussed other methods for testing the toxins, and dose finding that would be less invasive. The Committee discussed the study design in detail, and the application for the work being done by the group. The Committee discussed the possibility of group housing the animals and the pros and cons for this option.

Vote on the Motion: The Significant Change was approved with 11 members voting in favor and 1 member opposed.

Following the vote, the Committee decided to ask the PI to run the brain cholinesterase assay on the animals that die. Then if there are further developments of this work they would have that data.

- **Dr. Grue, 2185-47, “Procellacor and Chinook Salmon” Significant Change**

AS presented a summary of an amendment for IACUC protocol #2185-47 *Procellacor and Chinook Salmon*. This amendment is being reviewed by the full committee because it involves the implementation of a functional predation test.

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Background:

The goal of this protocol is to evaluate the safety of a new herbicide, Procellacor, to juvenile Chinook salmon in order to facilitate the state permitting and federal registration process. Procellacor represents a promising new class herbicide, with low application rates, short contact times and an excellent environmental profile. For example, toxicity of the formulated product to the common carp and juvenile rainbow trout is low relative to the maximum proposed application rate in the field. However, one of the targeted use areas is the Pacific Northwest, and the associated states, including Washington, and the National Marine Fisheries Service (NMFS) require additional data on the toxicity of Procellacor to ESA-listed salmonids (e.g., Chinook salmon, *O. tshawytscha*) to support a state NPDES (National Pollutant Discharge Elimination System) permit.

Completion of the first of two standardized toxicity tests with the herbicide (already approved on the protocol) demonstrated no overt toxicity in juvenile Chinook exposed to 2x the maximum proposed application rate for 96 hours. NMFS has recommended that, as part of the standardized toxicity testing, the group obtain data to determine whether or not the herbicide exposed fish suffer any subtle or sublethal effects that may reduce survival.

Summary of this significant change:

The proposed study being added with this amendment requests the use of a functional predation test using smallmouth bass and Chinook smolts. Notably, the predation of juvenile Chinook by smallmouth bass is a natural occurrence in Lake Washington and elsewhere in the Pacific Northwest. This test will allow the group to determine if exposure to herbicide results in sublethal effects that may impair predator avoidance. Briefly, the study will involve 20 Chinook smolts (10 herbicide exposed, 10 controls) being exposed to 2-4 small mouth bass for 48 hours in an arena that contains flowing Lake Washington water, cobble substrate and artificial vegetation. This test will be replicated 6 times using new smallmouth bass and Chinook smolts for each replicate. It is anticipated that up to 50% of the Chinook will be consumed by the bass.

Both smallmouth bass and Chinook smolts will be conditioned prior to testing to simulate the natural behaviors/capabilities of both the predator and the prey. Smallmouth bass utilize a number of different prey in Lake Washington and Lake Sammamish, and therefore will need to be conditioned to Chinook smolts as their sole source of food. Similarly, Chinook smolts will be acclimated to the olfactory cues of conspecific predation as the hatchery Chinook smolts are naive relative to predation. Similar conditioning procedures have been used in other predation studies to help ensure predator/prey interaction will occur.

The information requested by NMFS to ensure the safety of the new herbicide to Chinook requires a "realistic" environmental setting and exposure scenario. The group believes the functional predation test is the best way to assess whether or not herbicide exposure may have sublethal effects in Chinook. Functional predation tests provide the advantage of incorporating the natural behaviors and capabilities of both the predator and the prey within a more natural setting, compared to studies of individual parameters without environmental context (e.g., assessing physical ability by measuring swimming speed, or physiological effects by assessing olfaction). Without environmental context, the biological significance of differences in associated metrics due to herbicide exposure cannot be adequately evaluated.

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Dr. Grue, the protocol PI, is available to answer any questions or concerns that the committee may have.

The Committee discussed the study design, rationale for animal numbers, study conditions, e.g., water temperature and the possibility of alternative endpoints.

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

Discussion: None

Vote on the Motion: The Significant Change was approved unanimously with 12 members voting in favor.

- **Kojima, 4310-01 “A Neuronal Process of the Error Signal That Drives Saccade Adaptation” Significant Change**

JFI explained that this was request for a repair surgery for a non-human primate on a neuroscience study.

Background: This group studies the role of different brain areas in the adaptation of eye movements. Since different types of eye movements are generated by different parts of the brain, the knowledge gained from these experiments helps the neurologist diagnose which part of the brain is damaged based on the patient’s eye movement deficits. Eye movements can also be used to allow quantitative exploration of cognitive processes such as learning, memory, and decision making.

Amendment: In this amendment the group is requesting one repair surgery for A13002, a 7 year old male rhesus macaque. His first surgery on this protocol was in October 2014 to implant a left eye coil and head lugs. Animals on this protocol are implanted with three head lugs – one behind each ear for head stabilization, and one on the top front of the skull in association with the eye coil. These lugs are affixed to the skull with dental cement and small titanium screws. His front head lug sloughed off in December 2016, and an approved repair surgery was used on January 11th to replace the head lug, as well as remove the damaged left eye coil and implant a right eye coil. Having used that repair surgery, a new repair surgery was requested and approved at the January IACUC meeting. This animal then sloughed his back left head lug on February 7th, and on April 12th the approved repair surgery was used to replace that head lug. During the same surgical session, 3 recording chambers were implanted. There are no current concerns with these implants. The group reports that this animal has completed his behavior training, and is halfway through his behavioral experiments. His continued enrollment in this study is valuable due to both the time investment of training, as well as the need to acquire a sufficient amount of data to support publication.

Current implants: Right eye coil, three head lugs, three chambers.

Surgical history: This will be the third repair surgery requested for this animal, two having already been used. Other than the surgeries described above, this animal had 5 lymph node biopsies and 2 bone marrow collections in 2013 and early 2014 prior to his assignment to the current protocol.

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Clinical History: Seems to have recovered well from previous surgeries, and appears to be healthy. He did have some seizure activity in April thought to be associated with ketamine administration, but no seizure activity or deficits noted since.

Behavior Report: This animal is not a behavioral case. He is currently singly-housed because he was determined to be incompatible with his previous social partner, and was then kept in single housing for surgery and recovery. Behavior Management Services is in the process of identifying new potential partners.

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

Discussion: JM asked if there was any specific reason that the lugs fell off. CH explained that that there was not and that it just happens sometimes due to the nature of the implant.

Vote on the Motion: The Significant change was approved unanimously with 12 members voting in favor.

Approval of the IACUC Meeting Minutes

The IACUC Chair called for the approval of the April 20, 2017 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 7 members voting in favor and 5 abstentions.

OAW Director's Report

Noncompliance: UVB exposure

reported to OLAW 5/18/17.

Motion: to send letters of counsel PI & individual involved.

Discussion: Amendment to clarify the dose and increase the monitoring has been approved.

Vote: all in favor.

Attending Veterinarian's Report

Facility issues:

- Humidity: No issues reported.
- Temperature and lights:
4/10 and 4/15: Temperature in this decentralized facility housing mice fluctuated from 70-77. This has been corrected.

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4/25/17: Light did not come on in 4 animal holding rooms in this centralized mouse facility. In working up this issue, it was discovered there was a failure in a node that resulted in some rooms with the lights not turning off. DCM is working with the facility building system management team to determine a better way of alarming lighting in this facility. Talks are ongoing.

Protocol Monitoring:

There are 14 active protocols on the veterinary monitoring program. At this time they are all going well.

Adverse event:

An adult female mouse was found dead on 3/25/17 in a cage that had no cage card or food, and was put on the rack backwards. This was a rack with autowater, so the cage was not connected to water. This cage was a “set-up” cage that was on the rack for investigators to use if they needed a new cage. A necropsy was performed on the carcass. All organs were severely autolyzed so the cause of death was unclear. Subsequent investigation by VS and Husbandry with the investigative group in the room and the husbandry staff did not discover how the mouse came to be in that cage.

HBA Subcommittee:

The HBA committee met this month. There was a discussion of cases of morbidity and mortality. In addition, we discussed one protocol, Kean 4315-02 and reviewed the Harm/Benefit of this protocol.

Other Business:

The Committee discussed improvements to the WaNPRC break room as noted in a previous meeting. No definitive answer regarding funding for the noted issues, but it was thought that the use of the AAALAC budget was unlikely. The Committee discussed the possibility of citing the issues as part of a site visit.

Closing Business:

The Meeting was brought to a close at 5:09 pm. The floor was opened to public comment.