DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 480-3387

March 23, 2020

Re: Animal Welfare Assurance
A3248-01 [OLAW Case 1T]

Dr. Elizabeth Cantwell
Senior Vice President for Research
University of Arizona Health Sciences Center
P.O. Box 210066
Tucson, AZ 85721-0066

Dear Dr. Cantwell,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your March 20, 2020 letter responding to my February 18, 2020 request for information regarding an allegation of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the University of Arizona. According to the information provided, OLAW understands that the Institutional Animal Care and Use Committee (IACUC) and Biosafety Officer investigated the allegations and determined the following:

1) Investigational viruses and bacteria were approved to be injected by the retro-orbital route and by tail vein.

2) A minor deviation was identified whereby an anesthetic cocktail containing acepromazine was given to three mice for thymectomy. The approved protocol stated that ketamine/xylazine, without acepromazine, was to be used for this procedure.

3) A minor deviation was identified whereby three mice received the approved analgesics post-operatively rather than pre-operatively as per protocol.

4) Mice were appropriately monitored in accordance with the protocol while anesthetized for surgery.

5) In-date ophthalmic ointment and iodine surgical scrub was used for surgery.

6) All surgical assistants had been appropriately trained and used appropriate surgical garb.

7) Surgical instruments were initially autoclaved and then sterilized between animals with a bead sterilizer, which is consistent with institutional guidelines.

8) The surgical field was appropriately cleaned with chemical disinfectant before and after use.

9) Sterile saline was correctly used for specific surgical procedures.

10) Wound clips were removed at the approved timeline.

11) The Principal Investigator appropriately supervised the surgeries.

12) The Biosafety Officer indicated that all biohazards were handled in accordance with the protocol and regulations.

Based on its assessment of these explanations, OLAW understands that other than the two minor deviations (#2,3), the allegations were not substantiated. The deviations were appropriately addressed by the investigator. The IACUC may wish to consider greater flexibility in how protocols are written to include various choices such as the ingredients of anesthetic cocktails or the timing of analgesic administration. Because the complainant did not request follow up, no additional actions will be taken. OLAW hereby closes this investigation and thanks you and your staff for your prompt and thorough response.

Sincerely,

(b) (6)

Axel Wolff, M.S., D.V.M.
Deputy Director
Office of Laboratory Animal Welfare

cc: IACUC Chair
NIA Director, Division of Extramural Activities

THE UNIVERSITY OF ARIZONA
Research
Innovation & Impact

Administration 601
PO Box 210066
Tucson, AZ 85721-0066
Ofc: 520-621-3513
Fax: 520-621-7507
research.arizona.edu

March 20, 2020

Axel Wolff, DVM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
Rockledge 1, Suite 360
6705 Rockledge Drive
Bethesda, MD 20892

Re: A3248-01 [OLAW Case 1T]

Dear Dr. Wolff,

The University of Arizona, in accordance with Assurance D16-00159 (A3248-01) and PHS Policy IV.F.3., provides this follow-up incident report regarding this report of alleged noncompliance filed by (b) (6) (b) (6) This incident was first reported to Dr. Axel Wolff, OLAW, on or about 02/09/2020 via an email from (b) (6) of the Dr. Janko Nikolich-Zugich lab.

On 02/09/2020 the IACUC and the Attending Veterinarian received an email from (b) (6) (b) (6) with the same allegations that were submitted to NIH-OLAW. The IACUC reviewed the allegations at the 02/10/2020 IACUC meeting and formulated the approach to investigate the allegations. The IACUC requested documentation from Dr. Nikolich-Zugich, including surgical and training records. IACUC member (b) (6) who also has delegated veterinary oversight authority by the Attending Veterinarian, inspected the surgery areas utilized by Dr. Nikolich-Zugich, reviewed surgery records, and interviewed rodent surgeons/surgery assistants in the lab on February 21, 2020. The IACUC referred the allegation regarding inappropriate handling of West Nile Virus to UA (b) (6) for investigation. Dr. Nikolich-Zugich provided the surgical and training records and other information the IACUC requested for review, held a mandatory lab meeting to review the allegations with his staff, and provided a comprehensive response to address the allegations. The following summarizes the results of the IACUC investigation:

1) On Protocol 08-102 injection procedures on mice were different from what had been described in the approved protocol. The retro-orbital route had been used rather than tail vein for injection of an infectious agent.

- Unsubstantiated. Protocol 08-102 was amended in 6/6/19 to allow for bacterial and viral infections to be injected via RO sinus or IV (tail vein). Prior to this date, the approved route for injection of bacterial and viral infection was via IV in tail vein only.

2) Mice were anesthetized with a different agent than stated in the protocol.

- Minor deviation. Both Ketamine/Xylazine/Ace (KXA) and Ketamine/Xylazine (KX) cocktails are approved on the protocol. KXA is approved for parabiosis surgery. KX is approved for other surgeries, including thymectomy. Per surgery records KXA was used for 3 thymectomy surgeries, and KX was used for the other 9 thymectomy surgeries performed.

3) Analgesia was given at a different time than approved and at a different concentration.

- Minor deviation. Approved analgesics at approved doses were administered. Pre-operative analgesia, as described in the protocol, was administered to 9 out of 12 mice undergoing thymectomy. The remaining 3 mice were administered analgesia post-operatively.

4) Mice were not appropriately monitored while anesthetized.

- Unsubstantiated. Surgical records indicate that the animals were monitored for depth of anesthesia every 15 minutes throughout surgery, as described in the approved protocol in accord with IACUC rodent surgery guidance.

5) The surgical scrub used expired iodine, ophthalmic ointment was not used, and pre-emptive analgesia was not given.

- Unsubstantiated. Records indicate ophthalmic ointment was administered. No expired ophthalmic ointment was observed in the surgery room. No expired iodine was noted in the surgery room.
- Analgesia administration is addressed in item #3 response.

6) Surgical assistants had not been adequately trained and did not wear appropriate surgical garb.

- Unsubstantiated. Dr. Nickolich-Zugich, the surgeon, trains all staff who serve as surgical assistants, and all wear appropriate surgical garb.

7) Surgical instruments were used among several animals without sterilization.

- Unsubstantiated. Instruments are initially autoclaved and a bead sterilizer is used between procedures to re-sterilize instruments for up to 8 animals per pack, in accord with approved IACUC rodent surgery guidance.

8) There was inappropriate preparation of the surgical field.

- Unsubstantiated. The surgical field is cleaned using a chemical disinfectant spray before and after use. Devices are wiped with alcohol before and after use.

9) Sterile saline was not provided during surgery.

- Unsubstantiated. Sterile saline is listed as palliative support for certain surgery procedures and was administered appropriately for those procedures per surgery records.

10) The post-operative care did not include appropriate analgesia and wound clips were not removed until day 21)

- Unsubstantiated. Wound clips were removed on day 14 per interview and surgery records.
- Analgesia administration is addressed in item #3 response.

11) There was failure by Dr. Janko Nikolich-Zugich to adequately supervise the surgeries.

- Unsubstantiated. Dr. Nickolich-Zugich was the surgeon per interview and surgery records.

12) Samples of West Nile Virus were not handled in accordance with biosafety and select agent policies by (b) (6)

- (b) (6) conveyed in his report to the IACUC that "All of these mice were housed in Bio 5 (b) (4) and handled within certified biosafety cabinets using ABSL3 precautions. Some of these experiments did require that samples (blood, spleen, LNs) be collected and others were for mortality (we monitor the mice for signs of being moribund and then humanely

THE UNIVERSITY OF ARIZONA
Research
Innovation & Impact

Administration 601
PO Box 210066
Tucson, AZ 85721-0066
Ofc: 520-621-3513
Fax: 520-621-7507
research.arizona.edu

euthanize them when they meet that criteria). All samples collected were transported to the BSL3 in double containment and coolers. All processing took place within certified biosafety cabinets in the Keating 4th floor BSL3."

The IACUC reviewed and discussed all documentation generated by the investigation at the 03/09/2020 IACUC meeting. In summary, the IACUC and Biosafety Officer were unable to substantiate the allegations and found that work was conducted in accord with the approved protocols, except for the two minor deviations noted in items #2 and #3 above. Dr. Nikolich-Zugich has addressed these deviations to be compliant with his protocol to the satisfaction of the IACUC. The IACUC determined that this was not a programmatic failure and did not reach the threshold of non-compliance as the two minor deviations did not significantly impact animal welfare.

The University of Arizona is committed to protecting the welfare of animals used in research and appreciates the guidance and assistance provided by OLAW in this regard. Should you have any questions regarding this case, please contact Dr. David Besselsen, UA Attending Veterinarian, at (b) (6) or besselsd@email.arizona.edu.

Thank you for your consideration of this matter.

Sincerely,

(b) (6)

Elizabeth Cantwell, PhD
Senior Vice President for Research & Innovation
Institutional Official
The University of Arizona

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Monday, March 23, 2020 7:07 AM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: NIH OLAW Case A3248-01 Response - University of Arizona

Thank you for this report, (b) (6) We will send a response soon.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: (b) (6)
Sent: Friday, March 20, 2020 6:39 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Cantwell, Elizabeth R - (ecantwell) <ecantwell@arizona.edu>
Subject: NIH OLAW Case A3248-01 Response - University of Arizona

Dear Dr. Wolff,

On behalf of Dr. Cantwell, please find the University of Arizona's response to case A3248-01 attached.

Best regards,
(b) (6)

THE UNIVERSITY OF ARIZONA

(b) (6)

DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 480-3387

February 18, 2020

Re: Animal Welfare Assurance
A3248-01 [OLAW Case 1T]

Dr. Elizabeth Cantwell
Senior Vice President for Research
University of Arizona Health Sciences Center
P.O. Box 210066
Tucson, AZ 85721-0066

Dear Dr. Cantwell,

The Office of Laboratory Animal Welfare (OLAW) received, from a Program Officer at the National Institute of Aging, NIH, an allegation of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the University of Arizona which had been filed by (b) (6) The complaint contained few specifics and no supporting documents. Please direct the Institutional Animal Care and Use Committee (IACUC), avoiding any conflicts of interest, to investigate the alleged incidents and determine if they can be substantiated. If noncompliance is confirmed, please provide a reasonable and specific plan and schedule for correction. The items cited are as follows:

1) On protocol 08-102 injection procedures on mice were different from what had been described in the approved protocol. The retro-orbital route had been used rather than tail vein for injection of an infectious agent.

2) Mice were anesthetized with a different agent than stated in the protocol.

3) Analgesia was given at a different time than approved and at a different concentration.

4) Mice were not appropriately monitored while anesthetized.

5) The surgical scrub used expired iodine, ophthalmic ointment was not used, and pre-emptive analgesia was not given.

6) Surgical assistants had not been adequately trained and did not wear appropriate surgical garb.

7) Surgical instruments were used among several animals without sterilization.

8) There was inappropriate preparation of the surgical field.

9) Sterile saline was not provided during surgery.

10) The post-operative care did not include appropriate analgesia and wound clips were not removed until day 21.

11) There was a failure by Dr. Janko Nikolich-Zugich to adequately supervise the surgeries.

12) Samples of West Nile Virus were not handled in accordance with biosafety and select agent policies by (b) (6)

Please have the IACUC evaluate these allegations and provide an assessment by **March 25, 2020**. Feel free to contact me should you have any questions.

Sincerely,

(b) (6)

Axel Wolff, M.S., D.V.M.
Deputy Director
Office of Laboratory Animal Welfare

cc: IACUC Chair

A3248 - IT

Wolff, Axel (NIH/OD) [E]

From: (b) (6)
Sent: Sunday, February 9, 2020 3:33 AM
To: Janko Nikolich; (b) (6)
Cc: RLSS Help; UAC Veterinary Services; (b) (6) (b) (6) (b) (6)
Subject: IACUC protocol violations

Dear Prof. Dr. Janko Nikolich-Zugich,

Since you claimed that I never reported to you multiple violations of our protocols, biosafety and university polices I would like to remind you, as well as other internal and external offices, institutions and persons.

I will try to report you all of violations as witness as well as providing material evidence for all of my claims.

1. Violation of Biosafety Plan and mouse IACUC protocol

As I already reported (b) (6) violate Biosafety Plan - Research Laboratory and Safety Services (RLSS) and this plan addresses and meets the requirements of the Federal Select Agent Program. (b) (6) violated Biosafety Plan – RLSS since (b) (6) did not follow well define rules and did not treated samples from mice infected with Western Nile Virus (WNV) appropriately. I can witness all of this violations as well as I can provide material evidence that that is true. In March-May period last year I have done multiple experiments with (b) (6) using WNV infection as model. Since WNV virus is BSL3 lab pathogen all of harvest of samples from infected mice as well as processing samples should be done in appropriate BSL3 lab. (b) (6) violate well define rules multiple times since (b) (6) did not treated samples from mice infected with WNV appropriately. (b) (6) was taking unfixed samples outside BSL3 lab (potentially biohazard samples that contained WNV) in containers that are not leak proof (plates). That did not surprise me since she is doing that as well as other lab members since I joined lab in (b) (6). They usually fix the samples later because it is much easier for them to do the single sell suspensions staining ones they are in BSL2 lab space. They are covered since no one can claim that they did not follow rules because samples are fixed and ran on flow as fixed. Since I did not have a lot experience in BSL3 lab and (b) (6) was responsible for conducting that part of my experiments. I was part of all of this experiments (March-May 2017.) end I noticed that (b) (6) violate the biosafety rules and our IACUC protocols removing potentially biohazardous samples from WNV infected mice (unfixed) in plates (definitely not leak proof containers) outside BSL3 lab. Immediately after I notice this violation I reported that to (b) (6) (b) (6) respond me day later, telling me that (b) (6) test unfixed samples for WNV load, and (b) (6) convinced me that concentration of WNV is not enough to infect people working in lab (including students that sometimes are not covered with medical insurance). I was afraid, but since (b) (6) is most experienced BSL3 lab person as well as approval holder and person that was giving me a training and supervising me in BSL3 lab (b) (6) convinced me that I am not at risk to be infected. A while later (b) (6) went one step further and (b) (6) violated biosafety rules and mouse protocol again. In few experiments (b) (6) did not fixed samples at all and ran them on flow cytometer unfixed (b) (6) had done whole experiment: harvest of tissue from mice infected with WNV, getting single cell suspension from different tissues, staining with multiple vortex and flick steps and finally ran on flow cytometry – WITHOUT fixing samples). Since I was blocked from using our wiki page and since I did not have access to our data any more I made a copies for all claims and I would be more then welcome to provide it to you. The good thing is that our wiki page (laboratory notebook) remember all changes and as far as I know it is impossible to delete things (someone is trying to delete evidence but I have copies and print screens if someone is interested) Additionally, everyone with basic skills at flow cytometry can make a difference between fixed and unfixed samples (I can provide to you original data from this experiments to prove that samples from mice infected with WNV are ran unfixed including the presents of cells in samples that are positive for virus-tetramer positive cells). I do not expect that someone will do something about my claims at University level since so far

unfortunately everyone is so supportive for your inappropriate behavior. I had a moral obligation to point out biosafety violations of the protocol and now it is up to relevant internal and external institutions to investigate my claims.

2. Violation of IACUC protocol and inappropriate treatment of animals

Our IACUC protocol (# 08-102) was violated multiple time as I mentioned above. I will provide more evidence of violations with additional inappropriate treatment of laboratory animals. Multiple lab members that are using Listeria monocytogenes infection as model violate our IACUC protocol multiple times (every experiment that had done ones only one capable person to do tail vein i.v. injection left the lab at summer 2018. No one ever ask me for help in this part since I am capable to do I.v. tail vein injections).

Our protocol before 06/06/2019

"Intravenous (iv, RO, orbital): Mice (at least 8 weeks of age) in any experiment where an iv injection is referenced may receive this injection via retro-orbital route instead of tail vein, maximum volume 100uL. This procedure will be performed under isoflurane anesthesia. The exception to this route as an alternative to tail vein is viral or bacterial infection which will always be done by tail vein."

Our protocol after 06/06/2019

"Intravenous (iv, RO, orbital): Mice (at least 8 weeks of age) in any experiment, where an iv injection is referenced, may receive this injection via retro-orbital route instead of tail vein, maximum volume 100uL. Cells will be filtered or agitated prior to RO injection to prevent cell clumping. This procedure will be performed under isoflurane anesthesia. The exception to this route as an alternative to tail vein is injection of tumor cells."

The point is that all experiments and infections with Listeria monocytogenes (period from summer 2018. – summer 2019.) were done by multiple lab members (senior and junior) using retro orbital (RO) route. I can witness and also provide evidence that this is true. My claims were also confirmed by the fact that this has been discussed several times during laboratory meetings (ones there was long elaboration and presenting data from experiment that compared bacterial burden and load RO vs. I.V. tail vain), unfortunately only conclusion by you and laboratory manager was that this should be presented only as i.v. route without explanation that this was done RO (presented multiple times as RO route). I can understand why you was supportive for this violations. I guess that there are some consequences if the experiment are conduct contrary to the current approved protocols. I cannot understand who approved the amendment to our protocol (06/06/2019) that allowed use of RO route for infection (Hi vets, wasn't it weird that only mice in (b) (4) had eye problems in previous period?). Remember that this RO route is still used as a way of infection. Additionally, tree recently published papers (Listeria monocytogenes model) from our group in the methodology section claim that they were done in accordance with our approved protocols, which is obviously not true.

3. Violation of IACUC protocol and inappropriate use of FDA protected drugs

In a few of yours recently done surgeries (on one I was present) I notice multiple violations. Due to the lack of time since my official mail will be canceled today, I will only list the violations and will be available for any additional information.

- Inappropriate anesthesia (Inappropriate cocktail and ketamine concentration)

- Inappropriate analgesia (Inappropriate timing and concentration)

- Inappropriate anesthesia monitoring

- Inappropriate pre-operative procedures (surgical scrub with expired iodine, ophthalmic ointment was not used, analgesia was not provided during pre-op preparation)

- The first surgeon and assistants (some of them without appropriate training) did not follow the rule about surgical garb worn

- Surgical instruments were not sterilized appropriate (multiple use between animals without sterilization)

- Inappropriate preparation of surgical field, instruments and devices used

- Mice was not supported with sterile saline during surgery

- Inappropriate post-operative treatment (inappropriate analgesia and wound clips removed day 21)

All of this, in addition to the apparent absence of your surgical routine, explains why survival in full thymectomy surgeries in adult mice was less than 20% (By the way my survival in parabiosis experiments was more than 85% in adult mice). Multiple lab members were present and participate in this experiments and they can witness all that I mentioned here. I was just an observer and the idea was that I get training from experienced surgeon for this demanding surgical intervention.

4. Failure in your mentorship

I would like to mention here that the HR consultant concluded, after I reported your behavior, that there is obvious failure in leadership and supervision. Unfortunately, your behavior was supported by senior authorities and I was terminated for cause (failure to perform 😬) by the end as you know.

5. Violation of University polices

Just a reminder, you did not allow me to use sick leave (second time for 3 years period) even though I had proven (b) (6) (b) (6)

Whit this letter I have fulfilled my moral obligation to point your behavior and multiple violations done by you and other lab members, and for any additional information you can contact me on private e-mail (b) (6) as this one will be terminated soon.

If you think that someone else need to be included in communication please be free to forward my e-mail to all relevant addresses (NIH, FDA, Animal welfare organizations...)

Best regards,

(b) (6)

Get Outlook for iOS

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, February 12, 2020 10:36 AM
To: Santora, Kenneth (NIH/NIA/ERP) [E]
Subject: RE: Potential IACUC violation

Ok, thanks. I will start a general inquiry regarding the alleged animal care violations. I'll send you a copy of the case once it is closed.
Axel Wolff

From: Santora, Kenneth (NIH/NIA/ERP) [E] <kenneth.santora@nih.gov>
Sent: Wednesday, February 12, 2020 10:28 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Potential IACUC violation

Dear Axel,

All the information we received was in the email that was attached to my request. NIA's communication office was blind cc-ed on this email.

We do not have any information of the legitimacy of the claim or which, if any, grants are involved. This may be more relevant to NIAID?? Dr. Nikolich-Zugich is a PI with NIA in the University of Arizona with an active grants, but I cannot determine from the information provide if the complaint relates to one of these grants.

Ken

Ken Santora, Ph.D.
Director
Division of Extramural Activities
NIA/NIH/DHHS
Phone: 301-496-9322
Email: ksantora@nih.gov

From: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Sent: Monday, February 10, 2020 10:19 AM
To: Santora, Kenneth (NIH/NIA/ERP) [E] <kenneth.santora@nih.gov>
Cc: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Potential IACUC violation

Thank you for contacting OLAW about this matter, Dr. Santora. In order for us to proceed, please provide the following information:

1) What institution is involved?
2) Is this work funded by NIH?
3) Who is the PI on the grant under which this occurred?
4) Do you wish to be included in any correspondence between OLAW and the Institution?
5) Is the complainant wishing to be anonymous or can the name be used?
6) Do you have any other information as to how this was addressed by the institution?

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: Santora, Kenneth (NIH/NIA/ERP) [E] <kenneth.santora@nih.gov>
Sent: Monday, February 10, 2020 9:49 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Piazza, Geri (NIH/NIA/ERP) [E] <geri.piazza@nih.gov>; Kelley, Melinda (NIH/NIA/ERP) [E] <melinda.kelley@nih.gov>; McConnell, Cindy (NIH/NIA/ERP) [E] <mcconnellc@mail.nih.gov>
Subject: Potential IACUC violation

Division of Compliance Oversight:

Please see attached email correspondence from a scientist reporting lab violations.

I believe this should be followed up and investigated by your office. Let me know if I can be of further assistance.

Thank you,

Ken

Kenneth E. Santora, Ph.D.
Director
Division of Extramural Activities
NIA/NIH/DHHS
7201 Wisconsin Ave
Gateway Building Suite 2W113
Bethesda MD 20814
Phone: 301-435-2449
Email: ksantora@nih.gov