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) 4803387

January 16, 2020

Re: Animal Welfare Assurance
#A3095-01 (OLAW Case I]

Chris Kevil, Ph.D.
Vice Chancellor for Research and Director
Center for Cardiovascular Diseases and Sciences
Louisiana State University Health Sciences Center Shreveport
1501 Kings Highway
Shreveport, LA 71103

Dear Dr. Kevil,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your January 7, 2020 letter responding to OLAW's December 17, 2019 request for additional information regarding an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at Louisiana State University Health Science Center Shreveport.

According to the information provided, OLAW understands that all animals involved in the non-compliance were euthanized. There were no previous reports of issues involving the MCAO surgery and since the incident, the entire laboratory group has completed required training including hands-on training conducted by the Associate Director (AD). The principal investigator informed the Director and AD that all corrective actions have been implemented including placing feed and water (gel pack) on the floor to improve animal access post MCAO procedure and post-MCAO monitoring has been increased. Additionally, OLAW understands that any individual listed on protocols are required to complete an online training course and for those individuals conducting non-survival surgery, the AD reviews proper technique for non-survival surgery and institutional policy. For individuals conducting survival surgery, the AD conducts a hands-on lab including sterile technique (if applicable), proper anesthesia, proper use of analgesia and post-operative monitoring. Additionally, the AD observes the first survival surgery or surgeries by the individual. Post-operative care including monitoring of animals by the surgeon, is monitored by the Veterinary Services staff once the animals are returned back to the animal facility. A formal post-approval monitoring process has recently been instituted including visits to labs, observing surgical procedures and reporting the findings to the IACUC.

OLAW appreciates the prompt consideration of this matter by Louisiana State University Health Science Center Shreveport, which is consistent with the philosophy of intuitional self-regulation. Based on the information provided, OLAW is satisfied that appropriate steps have been taken to investigate this incident. We appreciate being informed of this matter and please contact us with any further questions or concerns.

Sincerely

Nicole Lukovsky-Akhsanov, DVM, MPH, DACLAM
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC Contact

LSU Health
SHREVEPORT

Health Sciences Center

Office of the Vice Chancellor for Research

Chris Kevil, PhD

1501 Kings Highway
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Shreveport, LA 71130-3932

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January 7, 2020

Nicole Lukovsky-Akhsanov, DVM, MPH, DACLAM
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
6700B Rockledge Drive, Suite 2500, MSC-6910
Bethesda, MD 20892-6910

Re: Animal Welfare Assurance #A3095-01 (OLAW Case I)

Dear. Dr. Akhsanov,

Thank you for the opportunity to offer clarification on the issue of non-compliance reported to you on November 19, 2019.

In response to your questions in your letter of December 17, 2019, I am providing the following additional information:

1. What is the disposition of the ten animals impacted by the non-compliance? If the animals were euthanized, please clarify your response as there is no further information provided.

All animals involved in the non-compliance were euthanized that day.

2. Has the IACUC or PI investigated concerns related to the MCAO procedure and the animals' accessibility to food and water?

Prior to this incident, after the animals had undergo the MCAO surgery, the animals were recovered in the lab overnight and subjected to the SPECKLE procedure the next morning. Animal Resources, and specifically Veterinary Services never saw these animals nor had any reports of issues involving this surgery. Based on our observations of this procedure done by other labs, which have been very successful, we had no reason to think there would be any issues. This non-compliance was discovered due to a mouse that was inadvertently left in a cage to be washed.

Typically, the animals on this protocol do not survive longer than 20 hours before the experiment is completed, and the animals are euthanized. Many of the other labs in this institution perform this MCAO as a non-survival procedure. No other lab conducting this MCAO procedure as a survival has been found to have the issue of accessibility to feed and water.

Since the incident, the responsible individual (as well as the entire lab group) has undergone the required training, including the hands-on training conducted by the Associate Director.

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December 17, 2019

Re: Animal Welfare Assurance
#A3095-01 (OLAW Case I]

Chris Kevil, Ph.D.
Vice Chancellor for Research and Director
Center for Cardiovascular Diseases and Sciences
Louisiana State University Health Sciences Center Shreveport
1501 Kings Highway
Shreveport, LA 71103

Dear Dr. Kevil,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your November 19, 2019 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at Louisiana State University-Shreveport. Your letter supplements the information provided in a preliminary telephone report made on October 28, 2019. The study is not supported by PHS funding.

According to the information provided, OLAW understands that on September 24, 2019 a veterinary technician reported finding a live mouse in a cage on the dirty side of cage wash. The clinical veterinarian investigated and determined the individual responsible for the mouse and located that individual in a procedure room. Upon reviewing the information, the individual immediately realized she had mistakenly left the box with a mouse instead of the empty box. During this conversation, the veterinarian observed nine other mouse cages, each with a single mouse in a recumbent position, which she assumed to be euthanized however on closer inspection, it was found that mice were alive and had large open scalp incisions. When the veterinarian questioned the individual if the animals were anesthetized, the individual said the mice had only been anesthetized with isoflurane while on the imaging stage of the laser speckle and now no longer anesthetized. The individual informed the veterinarian that the incisions were for the blood flow imaging (speckle procedure) and that all animals were going to be euthanized at the same time when imaging was completed as she was only halfway through. The clinical veterinarian asked if the individual thought it was appropriate to have conscious mice with large open wounds rolling around in bedding for hours before euthanasia. Following discussion with the individual, the veterinarian met with the principal investigator (PI) concerning the findings. The PI had an approved animal use protocol that describes these procedures however the protocol stated "*We will be able to use isoflurane at this stage because Laser Speckle is considered as our terminal procedure and we have achieved the desired infarction after middle cerebral artery occlusion (MCAO) surgery..... These mice will be decapitated under anesthesia and the brain will be used for TTC staining*". From the description in the protocol, the mice were to be decapitated under anesthesia. Several specifics issues were identified including:

- All the mice observed by the veterinarian did not have the speckle procedure performed at that point in time and mice did not appear ambulatory. Once caging was opened some mice did move, but movement was only to spin or circle which may have indicated an inability to obtain food and water following the MCAO procedure and will need more observation.

- The assembly line nature of the speckle procedure performed by the individual (10 animals in a row) allowed the individual to save time but disregard for welfare of mice. The veterinarian states she did not see the surgeries that were completed 24 hours prior but surmised that it would not be a stretch to think that 10 surgery in a row might lead to the same kind of disregards.
- When the individual left the procedure room with mice, the veterinarian states she assumed that the individual was taking the mice for euthanasia. In the discussion with the veterinarian, the individual states she returns the mice to the lab, puts the gas (CO2) on and removed the brain in the lab. The delay in euthanasia adds time to the period when mice have open incisions and no anesthetics or analgesia. In the chain of events as described, the speckle procedure is a survival surgery and not a terminal procedure. The scenario is not described in the approved protocol and is therefore a non-compliance.
- The preparation of mice for speckle procedure was performed without aseptic technique.

As a corrective measure, these are the recommendations of the veterinary staff:

1. The responsible individual has had prior non-compliance instances. Before being allowed to use animals again, all CITI training should be repeated, following by veterinary service specific training in sterile surgical techniques.
2. Surgeries and invasive procedures performed by the individual will be observed by veterinary services until veterinary services has confidence that the individual will adhere to approved protocol and is cognizant of animal welfare.
3. The individual is limited to performing a maximum of five surgeries in a single day and limited to Monday-Thursday morning- no surgery on Friday or weekends.
4. The individual will give veterinary services 24 hours advance notice of any plans to perform surgery or other invasive procedures.

The ACUC approved the recommendations and since the that time, the individual completed all training and conducted a surgical session under direct observation of veterinary services with the surgical session appropriately performed.

OLAW appreciates the prompt consideration of this matter by the Louisiana State University-Shreveport which is consistent with the philosophy of institutional self-regulation. Based on the information provided, OLAW requests further information as follows:

- What is the disposition of the ten animals impacted by the non-compliance? If the animals were euthanized, please clarify your response as there is no further information provided.
- Has the IACUC or PI investigated concerns related to MCAO procedure and animals' accessibility to food and water?
- Describe the required training for individuals planning to conduct surgery. How is proficiency verified before conducting survival procedures and how is post approval monitoring of those procedures conducted?

We appreciate being informed of this matter and look forward to your responses referencing **Case A3095-I** to the additional information request by **January 31, 2020.**

Sincerely,

(b) (6)

Nicole Lukovsky-Akhsanov, DVM, MPH, DACLAM
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC Contact

Report 1

LSU Health
SHREVEPORT

Health Sciences Center

Office of the Vice Chancellor for Research

Chris Kevil, PhD

1501 Kings Highway
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November 5, 2019

Brent Morse, DVM, Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
6700 Rockledge Suite 2500, MSC 6910
Bethesda, MD 20892

Dear Dr. Morse,

The LSU Health Sciences Center Shreveport (LSUHSC Shreveport), in accordance with Assurance D16-00059 (A3095-01) and PHS Policy IV.F.3., provides this report of a noncompliance issue.

The noncompliance issue is a result of a series of events involving one animal use protocol. A preliminary report was provided to you on October 30, 2019, via telephone call by Dr. V. Hugh Price, DVM, Director, Animal Resources and Attending Veterinarian. This report consists of the details of the non-compliance issue and was considered by the Animal Care and Use Committee (ACUC) of the LSUHSC Shreveport at its regularly scheduled monthly meeting on October 22, 2019.

The project and the animals covered by the animal use protocol that applies to this noncompliance issue are not on a PHS funded grant.

On September 24, 2019, the veterinary technician reported finding a live mouse in a cage on the dirty side of the cage wash. The clinical veterinarian investigated and determined the individual responsible for the cage and the mouse. She found that individual in a procedure room located on another floor. Upon stating the facts to that individual, that person immediately realized that she had mistakenly left the box with the mouse instead of the empty box when she went to the cage wash area.

While the clinical veterinarian was in the procedure room, she observed nine other boxes, each with a single mouse; most of these were recumbent, and she initially thought they were dead. On closer inspection, the mice were all alive and many had large open scalp incisions. She asked the individual if the mice were anesthetized. The individual told the clinical veterinarian that they had only been anesthetized (with isoflurane) while they were on the imaging stage of the laser speckle and that they were now no longer asleep. When the clinical veterinarian asked about the incisions, the individual said that was for the blood flow imaging (speckle procedure). Further, she stated that she was going to euthanize all the mice at the same time when she

had completed the imaging. At the point of this discussion, she stated that she was about halfway through. The clinical veterinarian asked the individual if she thought it was appropriate to have conscious mice with large open wounds rolling around in bedding for hours before euthanasia.

Following the discussion with that individual, the clinical veterinarian met with the principal investigator (PI) concerning these findings. The PI did have an approved animal use protocol that described these procedures. However, the protocol clearly stated, "We will be able to use isoflurane at this stage because Laser Speckle is considered as our terminal procedure and we already have achieved the desired infarction after MCAO surgery.... These mice will be decapitated under anesthesia, and the brain will be used for TTC staining". From the description in the protocol, the mice were obviously to be decapitated under anesthesia before they awoke from the speckle procedure.

Specific issues:

1. All of the mice observed by the clinical veterinarian had not had the speckle procedure performed at that point. The mice did not appear to be ambulatory. However, after the mouse cage was opened, some of the mice did move, but movement was only to spin or circle. This may have indicated an inability to obtain food and water following the MCAO procedure mentioned above, and will need more observation.

2. The assembly line nature of the speckle procedure performed by the responsible individual (10 mice in a row) allowed that individual to save time but ultimately led to a disregard for the welfare of the mice. The clinical veterinarian stated that she did not see the surgeries that were done 24 hours prior to this incident, but she surmised that it would not be a stretch to think that 10 surgeries in a row might lead to the same kind of disregard.

3. When the responsible individual left the procedure room with the mice, the clinical veterinarian stated that she made the assumption that the individual was taking the mice to a CO2 chamber for euthanasia. In the discussion with the clinical veterinarian, the responsible individual stated that she returns the mice to the lab, puts them under gas, and removes the brain in the lab. This delay in euthanasia adds time to the period when these mice have open incisions and no anesthetic or analgesic. Therefore, in the chain of events as described the responsible individual, the speckle procedure is a survival surgery and not a terminal procedure. The removal of the brain is a third procedure. This scenario is not described in the approved protocol, and therefore, are non-compliances.

4. Finally, the preparation of the mice for speckle procedure was done without any type of aseptic technique, compounding the problem.

Recommendations of the veterinary staff:

1. The responsible individual has had prior incidents with non-compliance. Before she is allowed to use animals again, all CITI training should be repeated, followed by veterinary services specific training in sterile surgical techniques.

2. In the future, surgeries and invasive procedures done by the responsible individual will be observed by veterinary services until veterinary services has the confidence that responsible individual will adhere to approved protocol and be cognizant of animal welfare.

3. The responsible individual will be limited as to the number of surgeries she may do in one day (five), and limited to Monday – Thursday mornings. Surgeries will not be done on Friday or on weekend days.

4. Finally, the responsible individual will give veterinary services 24 hours of advance notice of any plans to perform surgeries or other invasive procedures.

The ACUC approved the recommendations of the veterinary staff as a corrective action.

Since the ACUC met, the individual has completed all training and has conducted a surgical session under the direct observation of veterinary services. The surgical session was appropriately done.

No further action is necessary concerning this issue.

If additional information is required, please do not hesitate to contact me.

Sincerely,

(b) (6)

Chris Kevil, PhD
Vice Chancellor for Research
Dean, School of Graduate Studies
Institutional Official

Morse, Brent (NIH/OD) [E]

From: Morse, Brent (NIH/OD) [E]
Sent: Wednesday, November 06, 2019 2:03 PM
To: Price, Hugh
Cc: (b) (6)
Subject: RE: Report of Non-Compliance

Thank you for these reports Dr. Price. We will send official responses soon.

Best regards, Brent Morse

Brent C. Morse, DVM, DACLAM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: Price, Hugh [mailto:HPrice@lsuhsc.edu]
Sent: Wednesday, November 06, 2019 1:59 PM
To: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Cc: (b) (6)
Subject: Report of Non-Compliance

Dr. Morse,

Attached are two reports of non-compliance. Both reports were received by the Animal Care and Use Committee (ACUC) of the LSU Health Sciences Center at its regularly scheduled monthly meeting on October 22, 2019, and the recommendations listed in the reports from the veterinary staff were approved.

The responsible individual in report #1 has completed all requirements. The responsible individual in report #2 has not contacted the veterinary staff to begin re-training.

The ACUC considers both issues closed and has only requested it be notified when all individuals have completed the requirements listed in the recommendations.

If I need to provide additional details or information, please let me know.

Thank you.

Chip Price, DVM
Director, Animal Resources and Attending Veterinarian

A3095-I

Office of Laboratory Animal Welfare

OLAW

Initial Report of Noncompliance

By: [redacted] (b) (6)

Date: 10/28/19 Time: 4:15

Name of Person reporting: Chip Price DVM, A/V
Telephone #: [redacted] (b) (6)
Fax #:
Email:

Name of Institution: LSU HSC - Shreveport
Assurance number: A3095

Did incident involve PHS funded activity? No
Funding component: _____
Was funding component contacted (if necessary): _____

What happened? Mice waking-up under surgery. Surgery supposed to be non-survival but mice taken to lab & recovered.
Species involved: Mouse
Personnel involved: Grad. Student
Dates and times: 9/24/19
Animal deaths:

Projected plan and schedule for correction/prevention (if known): _____
Retrained lab + Grad Student
stopped work on protocol

Projected submission to OLAW of final report from Institutional Official:

OFFICE USE ONLY
Case #_____