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 9, 2020

Re: Animal Welfare Assurance
A3448-01 [OLAW Case 1D]

Bruce Doll, DDS, Ph.D., MBA
Assistant Vice President for Technology
Research and Innovation
IACUC Institutional Official
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814

Dear Dr. Doll,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your February 25, 2020 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the USUHS following up on an initial August 15, 2019 notification by email.

According to the information provided, this Office understands that the USU Animal Care and Use Committee (ACUC) determined that instances of noncompliance occurred with respect to: several incidents involving ferrets as the animal model for Blast + CHIMERA injury. The final report summarizes the events beginning with the first one that occurred on July, 31, 2019. One ferret was non-ambulatory following recovery from anesthesia. This was the 5th animal in a sequence of 8 that had a Blast + CHIMERA injury performed that day. The animal was given analgesics and monitored but eventually euthanized following veterinary consultation after exhibiting no improvement despite medical intervention.

On August 7, 2019 a ferret received a CHIMERA injury and recovered uneventfully with minor bleeding noted in the right ear. Per protocol, the animals underwent a follow up MRI the next day and there were no reported issues. A CT scan was performed on the same animal that afternoon and the isoflurane was set at 5% to keep the animal at the proper depth per the final report. After completion, it was noticed that the ferret had stopped breathing. Resuscitation was not successful, and necropsy revealed evidence of a stellate fracture of the right frontal bone.

On August 12, 2019 a ferret serving as a sham underwent isoflurane anesthesia to mimic conditions for the ferret from August 7th. This involved being in the induction chamber at 5% isoflurane flow rate for ~25min. While monitoring the animal, PI staff noticed that respirations had decreased drastically and removed the animal from the chamber. Resuscitation efforts by the veterinary staff were unsuccessful.

The last incident occurred on November 6, 2019. A single CHIMERA injury was performed on 8 animals. The second animal died immediately, and the veterinarian was called. It was hypothesized that individual variation might have made the animal more susceptible to the injuries. One more animal underwent the injury with the veterinarian present and recovered normally. However, the 6th animal had an adverse reaction and did not recover well per the report and was euthanized. At this point the CHIMERA injuries were halted and the remaining two animals did not undergo the procedure. A CT scan performed on the 2 animals that died, revealed skull fractures from the CHIMERA. The living animals were scanned and two had evidence of injury while the others had potential small skull abnormalities.

A subcommittee was created and an investigation occurred. The IACUC made the following recommendations listed below.

- The PI is working with the creator of the CHIMERA device to get a protection device that can be used in ferrets. This protection device should be implemented as soon as possible.

- The PI and staff have already implemented checking calibration on the CHIMERA device prior to beginning procedures each day. Recommend that this continues throughout the duration of the use of the equipment to ensure that proper forces are being used.

- The anesthetic tubing in the CT area has been inspected and replaced. Recommend adding a beginning of the day inspection checklist to the imaging SOPs to verify that all anesthetic equipment is inspected daily. Also recommend adding to the SOP that if isoflurane needs to be kept at 5% for longer than 2min during imaging, that a veterinarian is called to evaluate the situation.

- It was recommended to the PI, and she has agreed to implement, that all sham animals be removed from the induction chamber within 5min of initial induction and maintained under anesthesia via a nose cone.

- Recommend that the PI submit a modification to allow for a 10% mortality rate due to the injury. Due to the unexpected nature of head injuries, it is not unreasonable to expect that some animals may not survive.

- There are currently no activities being done on the protocol at this time. However, the recommendation is that when the next cohort begins, a veterinarian is present to observe the injury and anesthetic events to ensure that all corrective measures have been performed.

The PI agreed to all recommendations put forth by the IACUC. Per the report, a veterinarian was present to oversee all procedures and no complications have occurred and a team of IACUC members completed post approval monitoring of the final procedure and reported that everything went accordingly. It is stated that if the PI plans to use the CHIMERA equipment in the future, the IACUC has agreed that the PI will need to submit a brand new protocol after she meets with the AV and other IACUC members prior to starting any experiment to discuss her current protocol, modifications and potential alternatives.

It is noted that the final report did not include the funding source involving the incident. In the future, please include the source of funding and provide grant numbers for all PHS or NSF supported work. Based on the information provided, the Office of Laboratory Animal Welfare (OLAW) is satisfied that appropriate actions have been taken to investigate, correct, and prevent recurrence of the noncompliance. Please provide an update to OLAW on the status of the study if the PI resumes this type of research in the future. We appreciate being informed of these matters and find no cause for further action by this Office.

Sincerely,

(b) (6)

Jacquelyn T. Tubbs, DVM
Veterinary Medical Officer
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC Contact

Tubbs, Jai (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, February 26, 2020 1:46 PM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

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

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

From: (b) (6)
Sent: Wednesday, February 26, 2020 1:04 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; DHA NCR Reg Support Mailbox R2O2 <dha.ncr.reg-support.mbx.r2o2@mail.mil>; USARMY Ft Detrick MEDCOM USAMRMC Other ACURO <usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil>
Cc: (b) (6) Bruce Doll <bruce.doll@usuhs.edu>; iacuc@usuhs.edu
Subject: Re: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Hi Dr. Morse,

Please see the attached final report signed by the Institution Official. Feel free to contact us if you have any comments or concerns.

Thank you,
(b) (6)

Tubbs, Jai (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Tuesday, January 7, 2020 12:33 PM
To: (b) (6) OLAW Division of Compliance Oversight (NIH/OD); dha.ncr.reg-support.mbx.r2o2@mail.mil; usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil
Cc: bruce.doll@usuhs.edu; (b) (6) (b) (6) iacuc@usuhs.edu; (b) (6)
Subject: RE: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Good afternoon,

OLAW has not received a final report on this incident. Has progress been made? Please advise.

Thank you,

Jacquelyn Tubbs, DVM, DACLAM
Veterinary Medical Officer
Office of Laboratory Animal Welfare
National Institutes of Health

From: (b) (6)
Sent: Thursday, August 15, 2019 9:08 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; dha.ncr.reg-support.mbx.r2o2@mail.mil; usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil
Cc: bruce.doll@usuhs.edu; (b) (6) (b) (6) iacuc@usuhs.edu; (b) (6)
Subject: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Dear Dr. Morse,

Please find the attached initial incident report regarding a ferret protocol at the Uniformed Services University. A final report will be submitted by the IO once the investigation has concluded.

Thank you for your review.

(b) (6)

(b) (6)

[redacted]

Morse, Brent (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, January 16, 2020 3:54 PM
To: (b) (6) OLAW Division of Compliance Oversight (NIH/OD)
Cc: iacuc@usuhs.edu
Subject: RE: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Thank you for providing this interim report (b) (6) We will keep our case file open and await your final report. If necessary, please file another interim update in approximately 60 days.

Best regards, Brent Morse

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

From: (b) (6)
Sent: Thursday, January 16, 2020 11:09 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: iacuc@usuhs.edu
Subject: Re: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Good morning Dr. Morse,

The IACUC would like to provide an interim update on 08 August 2019 incident. Upon further discussion at the IACUC meeting on 15 January, the IACUC has chosen to continue the investigation of the incident. The PI has a pending modification with the IACUC and once it has been approved the PI will use her remaining 2 animals. The remaining procedure will be closely watched by the veterinarians and the committee. A final report will be sent at the conclusion of the procedure.

Thank you,
(b) (6)

On Wed, Jan 8, 2020 at 7:43 AM (b) (6) wrote:

> Good morning all,
>
> We discussed this incident at our last IACUC meeting in December and it was decided that another modification to the protocol has been requested. Therefore, this incident will not be closed out until the modification is processed and approved. We will keep you updated with the progress of this incident. Let me know if you have any other questions.

Thank you,
(b) (6)

On Tue, Jan 7, 2020 at 12:32 PM OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov> wrote:

Good afternoon,

OLAW has not received a final report on this incident. Has progress been made? Please advise.

Thank you,

Jacquelyn Tubbs, DVM, DACLAM

Veterinary Medical Officer

Office of Laboratory Animal Welfare

National Institutes of Health

From: (b) (6)
Sent: Thursday, August 15, 2019 9:08 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; dha.ncr.reg-support.mbx.r2o2@mail.mil; usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil
Cc: bruce.doll@usuhs.edu; (b) (6) (b) (6) iacuc@usuhs.edu; (b) (6)
Subject: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Dear Dr. Morse,

Please find the attached initial incident report regarding a ferret protocol at the Uniformed Services University. A final report will be submitted by the IO once the investigation has concluded.

Thank you for your review.

--

--

(b) (6)

[redacted]

August 15th, 2019

The IACUC was originally notified on August 8th, 2019 after communication between the PI and the veterinarian about an incident with several unexpected deaths in ferrets.

The first incident occurred on July 31st, 2019 during a sequential Blast + CHIMERA injury that was performed on 8 ferrets. The 5th ferret in the series was not ambulatory after waking up from the injury. The veterinarian was called and they agreed to watch the animal for several hours. As per protocol he was given pain medication. He was alert and eating baby food and moving his upper limbs. After 3 hours he was not fully ambulatory and we agreed in conjunction with a DLAR veterinarian to euthanize him. The other animals in the series (before and after this animal) did not have any problems.

The second incident on August 8th, 2019 and occurred while the animal received a CT scan. After removal from the scan and the anesthesia (5% isofluorane with a nose cone), the animal died after attempts at resuscitation and calling a veterinarian. This animal received a CHIMERA injury the day before but woke up quickly and was fully ambulatory and alert at that time, although there was slight bleeding from the nose. The CT was being conducted to determine the possibility of a skull fracture; there was a fracture. However, it is suspected that the calibration and dissemination of the anesthesia was not appropriate and this contributed to the death of the animal.

The third incident occurred on August 12th while a sham animal was anesthetized, to match the anesthesia being given to an animal receiving a CT scan. He was being monitored for his rate of breathing, but on removal from the chamber after several minutes stopped breathing. A veterinarian was called.

A subcommittee has been created and an investigation will occur.

A final report on the incident will be sent to OLAW when the incident has been resolved. Please contact us if you have any questions or concerns.

Contact person making report:

(b) (6)

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
OFFICE OF THE VICE PRESIDENT FOR RESEARCH
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799
www.usuhs.edu

25 February 2020

Brent Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
RKL1, Suite 360, MSC 7982
6705 Rockledge Drive
Bethesda MD 20892-7982

Dear Dr. Morse,

Final Incident Report
OLAW Assured Facility Number D16-00258 (A3448-01)

This is a final report on an incident at Uniformed Services University of the Health Sciences (USU) (OLAW Assured Facility Number D16-00258/A3448-01) that was initially reported to the Office of Laboratory Animal Welfare (OLAW) on 15 August 2019.

The USU IACUC was originally notified on 08 August 2019 after communication between the PI and the veterinarian about an incident with several unexpected deaths in ferrets.

On 31 July 2019, one ferret (the 5th animal in a sequence of 8 that had a Blast + CHIMERA injury performed that day) was non-ambulatory following recovery from anesthesia. After veterinary consultation, the animal was administered pain medication and monitored. Approximately 3 hours following the procedure, the animal was still not ambulatory and mentation was semi-comatose. The animal was euthanized and on necropsy a left frontal bone fracture was seen. (The other 7 animals who had the same injury done that day had no complications.)

On 7 August 2019, a ferret received a CHIMERA injury. Recovery, ambulation and mentation were normal, however, there was slight bleeding from the right ear. Following newly established protocol that all animals with evidence of bleeding have follow up imaging, the ferret underwent an MRI the morning of 8 August with no issues. A CT was then performed in the afternoon of 8 August to evaluate for a skull fracture. During the scan, the isoflurane was set at 5% in order to keep the animal at the proper depth. Monitoring parameters appeared normal during the entire procedure. After the CT was complete, as the ear tag was being replaced, it was noticed that the

ferret had stopped breathing. Resuscitation was not successful and on necropsy there was evidence of a stellate fracture of the right frontal bone.

On 12 August 2019, a ferret serving as a sham underwent isoflurane anesthesia to mimic the conditions for the ferret from 7 August, which involved being in the induction chamber for approximately 25 minutes at 5% isoflurane flow rate. While monitoring the animal, PI staff noticed that respirations had drastically decreased and removed the animal from the chamber and resuscitation was attempted unsuccessfully by veterinary staff.

On 6 November 2019, a single CHIMERA injury was performed (35PSI, no helmet) on 8 animals. There were no complications with the first animal; however, the 2nd one died immediately, so the PI called a veterinarian. This animal was slightly more lethargic than the others, so it was hypothesized that perhaps individual variation might have made him more susceptible to the injuries. Since the 1st animal recovered easily, and the previous group went without compilations at the same intensity, 1 more animal underwent the injury in conjunction with the veterinarian and recovered normally. The 6th animal had an adverse reaction and did not recover well. Following evaluation by a veterinarian, this ferret was euthanized. At this point the CHIMERA injuries halted and the final two animals did not endure the CHIMERA injury. A CT scan was performed on the 2 animals that died, which showed both had numerous, complex skull fractures from the CHIMERA. Necropsy was performed and histology to look for brain abnormalities or other causes was conducted. The living animals were scanned with CT 2 days later. Two of the animals showed injury while majority of the others have potential small skull abnormalities. A veterinarian radiologist was contacted to read the scans to determine if these are normal variations, which showed no other abnormalities were observed. The 2 animals that did not receive the 2nd CHIMERA did not show any obvious fractures. This suggests that the first round of CHIMERA did not cause skull fractures.

A subcommittee was created and an investigation occurred.

<u>The IACUC made the following recommendations:</u>

1. The PI is actively working with the creator of the CHIMERA device to get a protection device that can be used in ferrets. This protection device should be implemented as soon as possible.
2. The PI and staff have already implemented checking calibration on the CHIMERA device prior to beginning procedures each day. Recommend that this continues throughout the duration of the use of the equipment to ensure that proper forces are being used.
3. The anesthetic tubing in the CT area has already been inspected and replaced. Recommend adding a beginning of the day inspection checklist to the imaging SOPs to verify that all anesthetic equipment is inspected daily. Also recommend adding to the SOP that if isoflurane needs to be kept at 5% for longer than 2 minutes during imaging, that a veterinarian is called to evaluate the situation.
4. It was recommended to the PI, and she has agreed to implement, that all sham animals be removed from the induction chamber within 5 minutes of initial induction and

maintained under anesthesia via a nose cone. This will allow for additional monitoring (SpO2 and heart rate) in addition to respiration rate.

5. Recommend that the PI submit a modification to allow for a 10% mortality rate due to the injury. Due to the unexpected nature of head injuries, it is not unreasonable to expect that some animals may not survive. In rodent models using the same device, some deaths have been seen as part of normal operations, even though that is not the expected outcome.
6. There are currently no activities being done on the protocol at this time. However, the recommendation is that when the next cohort begins, a veterinarian is present to observe the injury and anesthetic events to ensure that all corrective measures have been performed.

The PI agreed to all recommendations put forth by the IACUC. A veterinarian has been present to oversee all procedures and no complications have occurred and a team of IACUC members completed a post approval monitoring of the final procedure and reported that everything went accordingly. If the PI plans to use the CHIMERA equipment in the future, the IACUC has agreed that the PI will need to submit a brand new protocol after she meets with the AV and any other IACUC members prior to starting any experiment to discuss her current protocol, modifications and potential alternatives.

The IACUC now considers this incident closed.

Sincerely,

(b) (6)

Bruce Doll, DDS, Ph.D., MBA
Assistant Vice President for Technology Research and Innovation
IACUC Institutional Official

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, February 26, 2020 1:46 PM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

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

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

From: (b) (6)
Sent: Wednesday, February 26, 2020 1:04 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; DHA NCR Reg Support Mailbox R2O2 <dha.ncr.reg-support.mbx.r2o2@mail.mil>; USARMY Ft Detrick MEDCOM USAMRMC Other ACURO <usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil>
Cc: (b) (6) Bruce Doll <bruce.doll@usuhs.edu>; iacuc@usuhs.edu
Subject: Re: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Hi Dr. Morse,

Please see the attached final report signed by the Institution Official. Feel free to contact us if you have any comments or concerns.

Thank you,
(b) (6)

A3448-10

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, August 15, 2019 10:53 AM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Thank you for this preliminary report. We will start a new case file and look forward to receiving the final report from the IO after the IACUC has completed its investigation.

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

From: (b) (6)
Sent: Thursday, August 15, 2019 9:08 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; dha.ncr.reg-support.mbx.r2o2@mail.mil; usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil
Cc: bruce.doll@usuhs.edu; (b) (6) (b) (6) iacuc@usuhs.edu; (b) (6)
Subject: Preliminary Report - USUHS - D16-00285 (A3448-01) 8 August 2019

Dear Dr. Morse,

Please find the attached initial incident report regarding a ferret protocol at the Uniformed Services University. A final report will be submitted by the IO once the investigation has concluded.

Thank you for your review.

(b) (6)

August 15th, 2019

The IACUC was originally notified on August 8th, 2019 after communication between the PI and the veterinarian about an incident with several unexpected deaths in ferrets.

The first incident occurred on July 31st, 2019 during a sequential Blast + CHIMERA injury that was performed on 8 ferrets. The 5th ferret in the series was not ambulatory after waking up from the injury. The veterinarian was called and they agreed to watch the animal for several hours. As per protocol he was given pain medication. He was alert and eating baby food and moving his upper limbs. After 3 hours he was not fully ambulatory and we agreed in conjunction with a DLAR veterinarian to euthanize him. The other animals in the series (before and after this animal) did not have any problems.

The second incident on August 8th, 2019 and occurred while the animal received a CT scan. After removal from the scan and the anesthesia (5% isofluorane with a nose cone), the animal died after attempts at resuscitation and calling a veterinarian. This animal received a CHIMERA injury the day before but woke up quickly and was fully ambulatory and alert at that time, although there was slight bleeding from the nose. The CT was being conducted to determine the possibility of a skull fracture; there was a fracture. However, it is suspected that the calibration and dissemination of the anesthesia was not appropriate and this contributed to the death of the animal.

The third incident occurred on August 12th while a sham animal was anesthetized, to match the anesthesia being given to an animal receiving a CT scan. He was being monitored for his rate of breathing, but on removal from the chamber after several minutes stopped breathing. A veterinarian was called.

A subcommittee has been created and an investigation will occur.

A final report on the incident will be sent to OLAW when the incident has been resolved. Please contact us if you have any questions or concerns.

Contact person making report:

(b) (6)