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
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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) 402-7065

December 26, 2018

Re: Animal Welfare Assurance
A4493-01 [OLAW Case C]

Ms. Sallie A. Houser-Hanfelder
Director
VA – Eastern Colorado Healthcare System
13611 East Colfax
Aurora, Colorado 80045

Dear Ms. Houser-Hanfelder,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your December 20, 2018 letter reporting an adverse event involving research mice at the Eastern Colorado Healthcare System Veterans Affairs Medical Center, following up on an initial report on November 7, 2018. According to the information provided, OLAW understands that 44% of mice on a lung cancer study died unexpectedly (or were euthanized) after receiving a urethane injection. All procedures were conducted in accordance with the protocol and no etiology was determined at necropsy. It is possible that this strain of mice was more prone to experiencing liver pathology and death following urethane injection or that there were circadian rhythm changes which altered the clearance of the drug from the body.

The corrective actions consisted of injecting the mice early in the morning to avoid circadian rhythm effects, injecting subcutaneous saline, amending the protocol to include potential morbidity/mortality, and specifying a specific strain of mice. The veterinarians will oversee the next injection, the injected agents will be tested to ensure safety, and the mice will be carefully monitored until fully recovered from anesthesia.

Based on its assessment of this explanation, OLAW understands that measures have been implemented to correct and prevent recurrence of this problem. OLAW concurs with the actions taken by the institution to comply with the PHS Policy on Humane Care and Use of Laboratory Animals. Please provide an update to OLAW following the next few experimental trails regarding the level of morbidity/mortality under the revised regimen. Thank you for keeping OLAW apprised on this matter.

Sincerely,

(b)(6)

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

cc: IACUC Chair
(b)(6) D.V.M., Ph.D., VA Chief Veterinarian
(b)(6)

Department of Veterans Affairs

Memorandum

Date: December 20, 2018

From: Director, Eastern Colorado Health Care System (554/00)

Subj: Reportable Incident Involving Loss of Animals in IACUC Protocol

To: Director, ORO Research Safety and Animal Welfare Workgroup (RSAW)

1. A reportable incident involving loss of animals was reported to me on December 19, 2018. In accordance with VHA Handbook 1058.01, this incident requires reporting to ORO within 5 business days after receiving the IACUC's notification.

2. The number and title of the study: IACUC #2M1803M: PPAR-Gamma Agonists for Lung Cancer Chemoprevention.

3. The funding source/sponsor for the study: VA Merit.

4. The incident was brought to the attention of the RMRVAMC IACUC on November 1, 2018. VA ORO and ORD, Medical Center Director, Chief of Staff, OLAW, AAALAC and the full IACUC were notified of the pending investigation on November 7, 2018. The VA IACUC met on November 13 and began initial discussion and full investigation.

5. The following describes the event and findings associated with this unexpected incident: Mice associated with this incident are FVB/N wild type and PGIS transgenic strains. Mice (n=63) were injected per protocol and urethane SOP, 2018-017 on Monday 10/29/2018. 28/63 (~44%) mice were FDIC (found dead in cage) or euthanized due to reaching protocol endpoints as of 11/13/2018. No predilections towards age, gender, weight, or transgenic vs wild type.

6. Diagnostic and necropsy results did not reveal any etiologies related to experimental procedures, and at this time, the IACUC are not 100% certain why this high mortality/morbidity occurred. Per the IACUC Veterinarian, the main differential is that it is a strain specific reaction to the urethane injection. FVB/N mice have strain specific liver pathology as described by Goelz et al. 1998. *Neuropathologic Findings Associated with Seizures in FVB Mice*. This underlying pathology may exacerbate or decrease clearance of urethane leading to toxicity and liver disease. Injections on this cohort occurred later in the morning than normal. Another differential is that there was altered clearance in these mice due to circadian rhythm alterations in clearance of urethane.

7. The IACUC believe the procedure, technique, and urethane quality were not an issue due to following the IAUC urethane injection SOP.

8. At the IACUC convened meeting held December 11, 2018, the Veterinarian presented his medical findings related to this incident. After the IACUC's review and discussion, the IACUC determined this incident to be reportable. This reportable incident will also be reported to OLAW, AAALAC, and a courtesy copy sent to ORD.

9. The IACUC has recommended to the PI the following to help prevent future unexpected outcomes associated with urethane injections in FVB mice:

   a. Amendment to protocol 2MR1803M
      1) Mice will be injected with urethane prior to 10 am to limit circadian rhythm variability
      2) Mice will receive 1 mL subcutaneous saline the day of injection
      3) Add an expected mortality and morbidity to the protocol for FVB mice injected with urethane (up to 45%)
      4) Justification for continual use of FVB mice over other strains of mice in this model

   b. Post approval monitoring
      1) Veterinarians will be present at the next urethane injection in FVB mice. Although not expected to be the issue, cultures and pH testing of urethane will be conducted to ensure product safety.
      2) Mice will be monitored post injection. Depth of sedation and survival will be monitored with the hypothesis that mice that are more sedated post injection will have higher incident of morbidity and mortality due to decreased clearance of urethane.

10. If there are any questions or areas to discuss, please contact (b)(6) at (b)(6)@va.gov or (b)(6) or (b)(6) Research Compliance Analyst at (b)(6)@va.gov or (b)(6)

(b)(6)

Sallie A. Houser-Hanfelder, FACHE
Director

Morse, Brent (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, December 20, 2018 3:26 PM
To: (b)(6) OLAW Division of Compliance Oversight (NIH/OD); accredit@aaalac.org
Cc: (b)(6) PhD; (b)(6)
Subject: RE: Incident

Thank you for providing this final report. We will send an official response 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: Armstrong, Teri L (b)(6) @va.gov]
Sent: Thursday, December 20, 2018 2:03 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; accredit@aaalac.org
Cc: (b)(6) hD (b)(6) @va.gov>; (b)(6)
(b)(6)
Subject: Incident

On November 7, 2018 I notified you that the VA-Denver (OLAW D16-00758/AA93-01), AAALAC VA-025, VA-554) was investigating a potentially reportable incident. The VA IACUC has concluded their investigation and determined that the incident was reportable. This study is being performed with VA grand funds. I am attaching the report that has been submitted to the Office of Research Oversite (ORO RSAW) for the VA.

Please let me know if you have any concerns or questions.

Teri

(b)(6)
VMU Supervisor/IACUC Director
Rocky Mountain Regional VA Medical Center
13611 E Colfax Ave.
Building B – RES 151
Aurora, CO 80045

(b)(6) Office
Cell

A4493-C

DEPARTMENT OF VETERAN AFFAIRS

MEMORANDUM

Date: November 7, 2018

From: (b)(6) IACUC Chair

Subj: Intent to Investigate

To: Medical Center Director. Research Compliance Officer, ACOS for R&D, CVMO, ORO, OLAW & AAALAC

It has been brought to the IACUC's attention that there has been an unanticipated loss of animal life on protocol 2MR1803M, specifically the portion of the protocol utilizing urethane to induce lung tumors. The IACUC, along with the veterinarian, animal care staff and lab staff will be investigating the cause of death in this study. All information will be discussed at the next convened IACUC meeting.

(b)(6)

IACUC Chair

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, November 08, 2018 6:50 AM
To: (b)(6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: VA Eastern Colorado D16-00748

Thank you for this preliminary report. We will open 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) @va.gov]
Sent: Wednesday, November 07, 2018 2:54 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: VA Eastern Colorado D16-00748

The IACUC at the VA in Aurora, CO is in the process of investigating a potential reportable incident.
Please see attached.

(b)(6)
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Rocky Mountain Regional VA Medical Center
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Building B – RES 151
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Cell