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

January 8, 2025

Re: Animal Welfare Assurance
A3281-01 [OLAW Case 3A]

P. Srirama Rao, Ph.D.
Vice President for Research and Innovation
Virginia Commonwealth University
800 East Leigh St.
Biotech One (b) (4)
Richmond, VA 23219

Dear Dr. Rao,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your final report, dated December 13, 2024, of an adverse event within the animal care and use program at Virginia Commonwealth University. Initial notification was provided during a Zoom meeting on December 3, 2024. According to the information provided, OLAW understands that between July and September 2024, 26 frogs died, and 35 frogs were euthanized due to disease. On August 6, the investigator contacted the Attending Veterinarian (AV) to discuss the issue. Further investigation revealed that the recirculating system's UV light filter was inoperable. Replacement parts were ordered and installed on August 8-9, 2024, along with fresh charcoal and refreshed crushed coral. Results from testing at Charles River Laboratories identified the presence of multiple pathogenic bacteria. Additionally, Chytrid fungus (Batrachochytrium dendrobatidis) was detected. The entire colony was euthanized upon AV recommendation, and the system was sterilized.

Preventive actions:

1. A new monitoring sheet has been developed to include checks for UV bulb functionality and frog health.
2. New training will be implemented for all animal technicians, covering animal handling, health surveillance, and system monitoring (including UV bulb maintenance).
3. A new form has been introduced for reporting frog illnesses or deaths.
4. New signage has been installed, providing on-call veterinarian contact numbers and a protocol for addressing sick or deceased frogs.
5. PI will conduct a weekly inspection of the system and frog health and submit monthly reports to the AV.

Based on the information provided, OLAW is satisfied that appropriate actions have been taken to investigate this incident, provide corrective measures, and prevent recurrence. OLAW concurs that the incident warranted reporting. OLAW recommends re-consideration if the two-day separation period during quarantine is sufficient. We appreciate being informed of this matter and find no cause for further action by this office.

Sincerely,
BRENT C. MORSE -S
Digitally signed by BRENT C. MORSE -S
Date: 2025.01.08 10:04:21 -05'00'
Brent C. Morse, DVM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC contact
(b) (6)

A3281-3A

Virginia Commonwealth University

Office of the Vice President for Research and Innovation

BioTech One (b) (4)
800 East Leigh Street
Box 980568
Richmond, Virginia 23298
(b) (6)

research.vcu.edu

P. Srirama Rao, Ph.D.
Vice president for research and innovation

December 13, 2024

Brent Morse, D.V.M., Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
(301) 496-7163

VCU Animal Welfare Assurance number D16-00180 (A3281-01)

Dear Dr. Morse:

Virginia Commonwealth University, in accordance with Assurance D16-00180 (A3281-01) and PHS Policy IV.F.3., submits this report of an adverse event involving the death of 26 frogs due to disease, and the euthanasia of 35 frogs between July and September 2024. This adverse event was previously discussed and reported during a Zoom meeting on December 3, 2024. During the discussion, you requested information regarding the "quarantine" process. The protocol includes provisions for quarantine and temporary housing, as outlined below:

- Frogs may be housed separately if their skin becomes irritated due to handling or shipping.
- Frogs received from the supplier will be housed separately for two days after arrival to minimize travel stress and monitor for obvious signs of disease before integrating them into the colony.

Temporary Housing Setup:
Frogs are placed in 10-gallon tanks with power filter systems rated for 30 gallons, providing quiet and efficient filtration. These systems include mechanical filter pads, activated carbon, and ammonia-removing media. Crushed coral in a mesh bag is used to maintain pH levels, and water conditions are monitored and maintained per established protocols.

Description of the Adverse Event:

On October 11, 2024, the investigator reported an adverse event involving the death of 26 frogs from disease, and the euthanasia of 35 frogs between July and September 2024.

In July of 2024, an abnormal increase in frog deaths was observed within the colony. Typically, the lab might experience one frog death per month, often attributed to age. While a single frog's death in June was not deemed unusual, four frogs died in July, marking the highest number of deaths in a single month since the colony was established.

The animal care technician, responsible for the daily care, water changes, and monitoring of the water chemistry of the system, did not observe outward signs of disease when discovering the dead frogs in July. The technician initially attributed the deaths to age. Water parameters were normal, and the same work-study student who had cared for the frogs during the fall of 2023 and spring of 2024 terms continued to do so.

In late July, the investigator conducted a more detailed examination of the frogs and their recirculating system. During this inspection, it was noted that the frogs exhibited increased skin shedding, and some displayed mild skin whitening observable only when handled. Additionally, some frogs appeared lethargic and less active than usual. On August 6, the investigator contacted the Attending Veterinarian (AV) to discuss the issue.

Further investigation revealed that the recirculating system's UV light filter, which is crucial for disinfection, was inoperable. The fault was not visible without a detailed inspection. Upon dismantling the UV bulb casing, the investigator found that the bulb had burned through its housing. Replacement parts were ordered and installed on August 8-9, 2024, along with fresh charcoal and refreshed crushed coral.

The AV subsequently collected and sent samples (preserved carcasses and feces) to Charles River Laboratories for analysis. While preparing the carcasses for shipping, a necropsy was carried out on two freshly euthanized frogs selected by the Principal Investigator (PI) as frogs she felt were showing signs of disease. No gross issues were noted by the AV either externally or internally. The only gross observation was that the ovaries appeared small and underdeveloped.

Despite these interventions, frog deaths continued. There were 11 deaths in August and 10 more in the first two weeks of September. By September, some deceased frogs displayed clear signs of disease, including white skin and increased frequency of shedding.

Results from testing at Charles River Laboratories identified the presence of multiple pathogenic bacteria. Additionally, Chytrid fungus (Batrachochytrium dendrobatidis) was detected in the carcass and the feces. This is a pathogen known to cause rapid colony decline, with symptoms such as excessive skin shedding and lethargy, aligning with earlier observations. No gross lesions or microscopic findings were noted from their examination of the provided carcasses. All results were based on PCR testing for prevalent infectious agents.

Immediate Actions Taken:

1. On September 12, 2024, the AV held a Zoom call with the PI and recommended euthanizing the entire colony and sterilizing the system. They discussed the ineffectiveness of available treatments, the challenges of eliminating the multiple bacterial and fungal species present in the system, and the potential for treatments to cause unintended death and suffering.
2. Following this consultation, all remaining frogs were euthanized during the last two weeks of September 2024. As of June 1, 2024, the system housed 61 frogs. Between June and mid-September, 26 frogs died from disease, and 35 were euthanized in September to prevent further suffering and contamination.

3. System sterilization began in October 2024 and is ongoing through November 2024. A published sterilization protocol, approved by the AV, is being used for this process.

Additional Actions:

1. A new monitoring sheet has been developed to include checks for UV bulb functionality and frog health. The UV bulb check includes inspecting the quartz sleeve and replacing components as needed. The sheet also includes a section for PI's weekly system inspections. DAR staff will verify that the monitoring sheet is properly completed.
2. New training will be implemented for all animal technicians, covering animal handling, health surveillance, and system monitoring (including UV bulb maintenance). This training will be documented, and the training records will be provided to the IACUC.
3. A new form has been introduced for reporting frog illnesses or deaths.
4. New signage has been installed, providing on-call veterinarian contact numbers and a protocol for addressing sick or deceased frogs.
5. PI will conduct a weekly inspection of the system and frog health and submit monthly reports to the AV.

The IACUC reviewed and discussed this adverse event and the associated actions during the full committee meeting on November 13, 2024.

We appreciate your consideration of VCU's efforts to prevent future incidents. VCU remains committed to animal welfare and acknowledges its ethical responsibilities in ensuring the well-being of research animals and complying with the PHS Policy on Humane Care and Use of Laboratory Animals.

Sincerely,

(b) (6)

P. Srirama Rao, Ph.D.
Vice President for Research and Innovation

Ware, Teagan (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, December 19, 2024 7:55 AM
To: (b) (6)
Cc: Srirama Rao; (b) (6) OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: [EXTERNAL] Follow-up letter (VCU Assurance# D16-00180/A3281-01)

Good morning,

Thank you for providing this final report for OLAW case **A3281-3A**. We will send an official response soon.

Best,
Teagan

Teagan Ware, MS, PMP
Animal Welfare Program Analyst
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

Phone: 301-435-2390
Email: teagan.ware@nih.gov

From: (b) (6)
Sent: Wednesday, December 18, 2024 4:30 PM
To: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>; OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Srirama Rao <psrao@vcu.edu>; (b) (6)
Subject: [EXTERNAL] Follow-up letter (VCU Assurance# D16-00180/A3281-01)

Dear Dr. Morse,

Attached is the follow-up letter signed by VCU's Institutional Official, Dr. Rao, who is copied on this email. During our Zoom meeting on December 3rd, I shared preliminary information regarding this issue. The attached adverse event report now provides a comprehensive overview of the situation and details the actions taken.

We sincerely appreciate your attention to this matter. VCU remains fully committed to compliance with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Guide, while maintaining our strong dedication to animal welfare.

If you have any questions or require further information, please feel free to contact me at (b) (6) (b) (6)

Thank you for your time and attention.

Best regards,

A3281-3A

Initial Report of Noncompliance

By: BCM

Date: **12/3/2024** Time: 10:30

Name of Person reporting: (b) (6)
Telephone #: (b) (6)
Fax #:
Email:

Name of Institution: __ Virginia Commonwealth Univ._____
Assurance number: ___A3281_____

Did incident involve PHS funded activity? __Yes____
Funding component: _____
Was funding component contacted (if necessary): _____

What happened: Pi contacted AV on August 6, 2024 about skin lesions in PI-maintained frog colony. UV light found not working. 10 deaths by end of August. CRL Path revealed "numerous bacteria, including red leg. Also, Chytrid fungus". AV recommended euthanizing colony. Total of 61 frogs died or were euthanized.

Species involved: Frog
Personnel involved: PI
Dates and times: 8/6/2024
Animal deaths: euthanized or died

Projected plan and schedule for correction/prevention (if known): _____

UV light repaired. Processes being updated and reviewed.

Projected submission to OLAW of final report from Institutional Official:

< 60 days.

OFFICE USE ONLY
Case #_____