



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

May 7, 2021

Re: Animal Welfare Assurance
#A3784-01 (OLAW Case D)

Dr. Karl Steiner
Vice President for Research
University of Maryland – Baltimore County
1000 Hilltop Circle
Baltimore, MD 21250

Dear Dr. Steiner,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your May 3, 2021 letter reporting an adverse event involving zebrafish at the University of Maryland- Baltimore County, following up on an initial report on April 9, 2021. According to the information provided, OLAW understands that approximately 1065 zebrafish died following a power outage which impacted the reverse osmosis (RO) system, the low water sensor, and the dissolved oxygen meter. The equipment failure led to pressurized air entering the water and causing fatal gas bubble disease in the fish.

The immediate action taken upon discovery consisted of moving live fish to a quarantine system and bringing in water from another building. The corrective actions consisted of repairing the RO and other components of the fish system. The fish breeding was temporarily halted to reduce stress in the breeding fish, the protocol was amended to include procurement of replacement fish from other laboratories, the laboratory staff was retrained on relevant standard operating procedures (SOP), and an emergency SOP was developed which addresses the RO system. A plan will be developed to address a source of emergency RO water, the RO system will be checked immediately after a power outage and records will be maintained, and users will be notified about RO problems to implement the emergency SOP.

Based on its assessment of this explanation, OLAW understands that measures have been implemented to correct and reduce the likelihood of a 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. As part of the development of the emergency plans, the institution also needs to address the emergency power supply to maintain critical services and support functions and consider alarm systems which can notify staff of any excursions from established parameters in the fish system. 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



Office of the Vice President for Research
University of Maryland, Baltimore County
1000 Hilltop Circle
Baltimore, Maryland 21250
phone 410.455.5636
research.umbc.edu

May 3, 2021

Assurance #: D16-00462

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

Dear Dr. Wolff,

On behalf of the UMBC Institutional Animal Care and Use Committee, and in accordance with PHS Policy IV.F.3., UMBC provides this report of noncompliance regarding a zebrafish loss to the Office of Research Protections and Compliance (ORPC) that was reported to our office by the PI at 7:28 A.M. on Wednesday April 7, 2021. This is a final report of the preliminary notification of the incident sent to the OLAW Division of Compliance Oversight by Andy Glenn on April 9, 2021, via email.

Noncompliance Report

On the evening of Sunday March 28, 2021, after a severe weather event, UMBC experienced a campus-wide power outage. This power outage caused the Reverse Osmosis (RO) water unit servicing the Biological Sciences building, which houses the zebrafish facility, to fail sometime between 7-11pm on March 28. As a result, the only remaining RO water was that which was left in the full tank. Unfortunately, the RO unit was not discovered offline until the morning of Tuesday March 30 during a routine building inspection by the building facilities manager. The RO system is maintained by the UMBC building manager and not the Principal Investigator (PI). The contractor that normally services the RO unit was notified but was unable to service the unit until Wednesday March 31.

The contractor notified the building manager on Wednesday March 31 that due to the extent of the damage to the RO unit caused by the power outage, the building RO unit would be down for several days as parts had to be ordered. The building manager contacted the PI to alert them of the situation in the afternoon of Wednesday the 31 around 4:30 p.m. While the RO unit was offline and not producing RO water, the building manager checked the RO tank twice a day and estimated the RO tank had sufficient water to meet the building needs through Friday April 2. During normal operation, the building manager checks the RO unit weekly for operational status.

On Friday April 2 in the morning, a staff member in the PI's lab checked the individual tanks water and logged that everything was normal. Friday afternoon the fish system was checked again by a PI's lab staff member. At this time the PI's staff member recorded a fish loss of over 50%. The staff member recorded that the pump was running dry and that there was no water running to the individual tanks. A check of the water pressure gage indicated there was no water pressure. The remaining live fish were immediately moved to a quarantine system in a fish room that had water flow. After conducting a health check on the remaining live fish, the PI's staff then checked the fish tank system and discovered that the water level in the fish tank system reservoir was too low to properly supply water to the individual fish tanks attached to the fish tank system. The water in the fish tank system reservoir is supplied by the building RO unit and by Friday afternoon the building RO stopped feeding RO water. The Biological Sciences building RO water tank was then replenished by transporting over 85 gallons from another building on campus. Once the fish tank system reservoir had been replenished, the water pump came back on and the system returned to normal operation. The Biological Sciences RO water unit was repaired on Saturday April 3 by the servicing contractor.

On Thursday April 8, a technician for Iwaki fish systems came to campus to make repairs on the fish system that were nonfunctional due to the power outage. This included repairing the low float level, flow switch and recalibration to the total gas pressure monitor.

The Iwaki technician agreed with the lab that the fish loss happened when the water levels fell too low (due to the RO water outage). The pump began to push air instead of water in the fish tank system reservoir. This pressurized gas was then distributed to each tank through the water pump which caused a buildup of gas in the water resulting in gas bubble disease among the fish.

The PI has an approved IACUC protocol to work with fish and has expressed remorse this unfortunate incident happened. The lab relies on embryos from the fish. The extensive death from the gas bubble disease caused stress in adult fish, and the PI expects this will impact egg production. The PI has put a pause on setting up breeding fish to allow them to recover from their high stress levels due to this problem. Some of the fish lost involved mutant lines used in the research. The PI stated these can be replaced by obtaining them from other labs and will submit a protocol amendment for IACUC approval. However, the PI's research will experience delays due to shipment from these labs and waiting for the fish to reach sexual maturity. Other stock was not lost entirely. These fish will need to be propagated and raised to adulthood (a few months).

IACUC review and discussion

The IACUC met on April 30, 2021 and discussed the reportable event. The PI in question is also an IACUC member and was recused from the discussion. The IACUC determined this was an isolated incident and not a programmatic failure. The IACUC also commented that this was a multi-system failure at the building and lab level. During the meeting, the IACUC accepted the corrective action submitted by the PI.

The IACUC specified the following corrective action plan:

1. All lab staff were retrained by the Principal Investigator to follow proper SOP to check water levels and document in the reservoir tanks of the fish system daily.
2. The PI has developed an emergency SOP to address RO issues for the fish facility to ensure proper function.
3. The University must take steps to develop a plan for emergency RO water. The IACUC advises that the PI work with UMBC Facilities Management, the department chair, and/or college dean to identify resources for the facility by either using building water piped through a charcoal filter directly into the reserve tanks or storing RO water in an emergency tank.
4. The building manager must check on the RO system immediately after a power failure to ensure its operational status. A record of this system check after each power interruption must be maintained.
5. The building manager must communicate with all RO users as soon as an RO water problem happens to enact the emergency RO SOP.

UMBC is committed to protecting the welfare of animals used in research and appreciates the guidance and assistance provided by OLAW in this regard. If you have any questions regarding this report, please contact Charles Bieberich, PhD, IACUC Chair.

With appreciation,

(b) (6)

Karl V. Steiner, PhD
Vice President for Research
University of Maryland, Baltimore County

cc: Charles Bieberich, PhD, IACUC Chair

(b) (6)

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, May 5, 2021 7:19 AM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Final Report of Noncompliance from UMBC

Thank you for this report, (b) (6) I will send a reply soon.
Axel Wolff

From: (b) (6)
Sent: Monday, May 3, 2021 2:04 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>; (b) (6)
(b) (6) Chuck Bieberich <bieberic@umbc.edu>; Karl Steiner <steinerk@umbc.edu>; Turhan Coksaygan <tcoksaygan@vetmed.umaryland.edu>
Subject: Final Report of Noncompliance from UMBC

Good Afternoon Dr Wolff; Deputy Director, OLAW

Attached please find the final report of noncompliance from UMBC. If you have any additional questions or concerns please feel free to reach out to me, Charles Bieberich, PhD, IACUC Chair or Turhan Coksaygen, DVM, our Attending Veterinarian.

Thank you for your time.

(b) (6)

(b) (6)

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Monday, April 12, 2021 7:13 AM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Preliminary Report of Noncompliance

Thank you for this comprehensive preliminary report, (b) (6) 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)
Sent: Friday, April 9, 2021 2:50 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: (b) (6) Chuck Bieberich <bieberic@umbc.edu>
Subject: Preliminary Report of Noncompliance

Good Afternoon

A principal investigator from our institution reached out to our office on 4/7/2021 with a reportable event. We wanted to make you aware of the issue and have provided a preliminary report of what happened.

- Name and contact information of person reporting (b) (6)
- Name of institution: University of Maryland, Baltimore County (UMBC)
- Assurance number : D16-00462 (A3784-01)
- Funding component and if contacted (for situations related to PHS-supported activities): NIH R21 HD 089476-01A1 (no cost extension) This grant is on a no cost extension and the bulk of the work for this funded proposal has been completed. Furthermore, the survival rate of fish stocks used for this study (AB and ndrg1a mutants), was relatively high, thus ongoing research will not be significantly disrupted. The PI therefore does not deem it necessary to inform the funding agency.
- Brief description of incident (e.g., species, category of personnel involved, dates, times, animal deaths):

Species: Zebrafish (*Danio rerio*)

Incident involving fish death: (informed from conversations with lab students and consultation with a technician from Iwaki Aquatic Service who inspected the facility on 04/08/21)

1. A power outage occurred on Sunday, March 28th (03/28/21) around 7 PM
2. The power outage resulted in lack of RO water production. The PI was only informed about the RO water problem on Wednesday, March 31st at 4 PM

3. During the time period between Sunday (03/28/21) and Thursday (04/01/21) evening, students in the PI's lab (both graduate and undergraduate researchers) used circulating water from the fish facility, unaware that there was a lack of RO water influx into the system and that this could seriously impact the proper function of the facility

4. A chain of events ensued linked both to the lack of RO water influx into the system and malfunction of two critical pieces of equipment in the fish facility, a valve that senses abnormally low water levels and a dissolved oxygen meter. These events played out as follows: The low water level was not detected due to malfunction of the valve that would have normally caused the pump to shut down. The pump continued to function, introducing highly pressurized air into the circulating water (as a consequence of the low levels of water in the system). Pressurized air is highly lethal to fish as it causes gas bubble disease. The system failed to detect the abnormally high levels of oxygen because the oxygen probe had not been regularly calibrated, resulting in a second failure of the pump to shut off.

5. On Friday morning, April 2nd the PI's student(s) found that many fish had died overnight – most likely as a consequence of gas bubble disease – the PI's student(s) also noticed that the pump was no longer working, due to a third malfunction, this time of a flow switch that caused intermittent loss of power to the system (pump and water dosing unit). This problem must have been linked to the power outage as the PI's lab has not had issues with intermittent power and pump shut down prior to that.

6. The dead fish were removed on Friday morning from the tanks and stored in the minus 80 freezer in the PI's lab in a sealed container. Water levels were replenished using RO water retrieved from the ILSB.

Personnel: PI, Fish technician, Graduate students, Undergraduate researchers.

Animal deaths: ~1065 adult fish died, an estimated 50% of the fish in the facility.

Plan and schedule for correction and prevention of fish death

1. On 04/08/21 an emergency servicing of the facility was carried out by Iwaki Aquatic Systems and a report from that visit will be forthcoming.

Pi will also develop a SOP to maintain the calibration of the oxygen probe with a retraining of PI staff to calibrate the probe based on the manufacturer specs.

2. The Standard Operating Procedure (SOP) for power outage is outlined below. The main difference with PI's prior SOP is that Pi and their staff will take the initiative to check with the facilities manager about the status of the RO water system following a power outage. The previous practice has been to wait until we were notified, which in this case was several days later. Pi will also retrain lab members on the SOP.

SOP for power outage:

1. Once there is a power outage, the pump immediately goes offline. This includes the main water pump and the peripheral pH/conductivity dosing pumps. This increases ammonia concentration and reduces water flow in the tanks. The first priority is to ensure water flow to the tanks. The electricity source for the pump is currently an emergency outlet.

2. Fish set-up/spawning activities and 10% exchanger will be halted IMMEDIATELY.

3. If the power outage persists longer than 12 hours without the pump functioning, PI's lab will begin manually replacing the water in the tanks.

4. Regardless of whether PI's lab is notified of an RO water outage, any time there is a power outage PI's lab will coordinate with the building facilities manager to check the RO water system.

5. PI's lab will obtain RO water from available buildings and keep several gallons as a backup to refill the reservoir as needed (2x the required amount will be kept on hand).

Timeframe for final report: 05/02/21- due to the IACUC meeting on 4/30/21 to discuss and approve the plan of correction.

Thank you

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(b) (6)