DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
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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

April 16, 2021

Re: Animal Welfare Assurance
A4051-01 [OLAW Case Z]

Dr. Wayne E. Cascio
Deputy Director, National Health and
Environment Effects Research Laboratory
U.S. Environmental Protection Agency
EPA-MD-B305-01, 109 T.W. Alexander Drive
Research Triangle Park, NC 27709

Dear Dr. Cascio,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your April 13, 2021 letter reporting an adverse event involving zebrafish at the US Environmental Protection Agency at Research Triangle Park, following up on an initial report on April 8, 2021. According to the information provided, OLAW understands that 100 zebrafish died after being transferred to a tank which had water contaminated with chlorine. It is possible that this occurred by splashing bleach water from a sanitizing dip tank onto equipment.

The corrective actions consisted of moving the dip tank to an area separate from stored fish equipment, washing fish tanks in a tunnel washer and rinsing with deionized water, rinsing twice with reverse osmosis water all equipment in contact with fish water, and storing clean tanks in the fish facility. A new fish equipment washer is being installed and this will allow greater separation of clean and dirty equipment. Additional washing and storage procedures will be implemented to further ensure this separation and prevent chemical contamination.

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.

Sincerely,

Axel Wolff, MS, DVM

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

cc: IACUC Chair

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CENTER FOR PUBLIC HEALTH AND ENVIRONMENTAL ASSESSMENT
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

April 13, 2021

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

Dear Dr. Wolff;

The purpose of this letter is to report an adverse event at the US EPA Research Triangle Park research program (Assurance # D16-00569, (b) (6) and to report on the measures being taken by the program to ensure another such event does not occur. A preliminary report of this event was emailed to OLAW by the IACUC Chair on Thursday, April 8, 2021.

The week prior to the event zebrafish fry had been transitioned from being reared in still plates in an incubator to being reared in tanks on a recirculating rack. Since the fry are very small initially, they are maintained in cylinders with screen bottoms in the tanks until they grow large enough to survive in the tank without added shelter or washing through the drain baffle on the tank. One of these tanks holding 4 cylinders of 19-day old zebrafish (100 total) had a mechanical failure where one of the cylinders fell over allowing the fry to escape into the larger tank. The fry were retrieved by netting and all fish in that tank were transferred to new cylinders and a new tank. Shortly after transfer the care staff checked on the animals and found all 100 animals in all cylinders in that tank dead. The situation was reported to the AV and the IACUC investigated.

Physical trauma is not suspected as all animals, including those who were not netted, were equally affected.

The aquatics specialist had been working offsite and measured the water quality parameters after returning to campus. The water quality parameters in the new tank were not markedly different than normal. It should be noted, though, that water quality parameters of that particular tank were not taken until between 1.5 and 2 hours after the fish died, and the tank was maintained on the rack with water flowing.

Due to the speed of death and the completeness of the deaths, the IACUC and animal care staff believe that the tank into which the zebrafish were transferred was contaminated with a residual chemical, most likely chlorine. This chemical would have been sufficiently diluted in the common sump water before recirculating to leave the rest of the fish on the rack unaffected. Further inspection of the room by animal care staff and the AV showed that clean equipment to

be used on the fish racks was stored adjacent to the bleach dip tank used to sanitize the aquatic equipment prior to washing. Splashing from the dip tank onto the waiting equipment would have been very easy.

It should be noted the dip tank is not usually kept adjacent to stored equipment. There is construction in the fish suite for the installation of a new, dedicated fish equipment washer, and the usual storage rooms are closed off and sealed behind plastic during this construction. Stored material and equipment are temporarily being held in the suite's common area, where some work has also been taking place.

The following corrective actions have been taken for the short term, during construction:
The dip tank is being stored in the janitorial closet and is used well away from clean equipment. Tanks and other equipment are then being run through a tunnel washer in central cage wash currently devoted solely to fish equipment, rinsed in deionized water, and stored in the fish facility. Clean equipment is stored away from all chemicals. All equipment that will be in contact with fish water is also now being thoroughly rinsed twice with reverse osmosis water prior to use to ensure surfaces in the aquatic environment are clean of any accidental deposits, be it chemical splashes or construction dust.

Once construction is complete there will be much greater separation of clean and dirty equipment. All dirty material will exit the fish suite from a rear door and be sent to the adjacent aquatic wash area. All washing of any sort will take place in this wash area which will have a wall and door separating clean and dirty sides. Reverse osmosis water will be incorporated as an automatic final rinse in the new, dedicated fish equipment washer. Clean caging will be dried on the clean side, away from possible contamination with washing or other chemicals, and stored either in clean side or in clean storage rooms in the fish facility also far away from chemical use areas.

We hope these measures satisfactorily address the issues. Please contact Steve Gavett (b) (6) (b) (6), Gavett.stephen@epa.gov) or Jaimie Graff (b) (6) Graff.jaimie@epa.gov), Animal Resources Program Office, if you have further questions.

Sincerely,

Wayne E. Cascio, M.D.
Director, CPHEA
Institutional Official

Stephen H. Gavett, Ph.D., D.A.B.T.
CPHEA / PHITD / CIB
IACUC Chair

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, April 14, 2021 8:03 AM
To: Gavett, Stephen
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Final report of adverse animal welfare incident and corrective actions

Thank you. We will send a response soon.
Axel Wolff

From: Gavett, Stephen <Gavett.Stephen@epa.gov>
Sent: Tuesday, April 13, 2021 9:00 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; Hoopes, Maria <Hoopes.Maria@epa.gov>; Jarrell, Leslie <Jarrell.Leslie@epa.gov>; Hedge, Joan <Hedge.Joan@epa.gov>
Subject: Final report of adverse animal welfare incident and corrective actions

Dear Dr. Wolff:

We have completed the final report on the deaths of 19-day old zebrafish which occurred at our institution (US EPA, Office of Research and Development, Research Triangle Park Program; OLAW Assurance number D16-00569, A4051-01) on 4/5/2021, as initially reported below on 4/8/2021.

The attached final report from our Institutional Official Dr. Wayne Cascio provides the details of the incident and the corrective actions which are being implemented.

Please let us know if you need any further information. We look forward to your response.

Sincerely,

Stephen Gavett, Ph.D., D.A.B.T.

Research Biologist
Cardiopulmonary and Immunotoxicology Branch
CIB PHITD CPHEA ORD USEPA
Tel. (b) (6)
Mailing Address:
U.S. Environmental Protection Agency
Attn: Stephen Gavett
Mail Code: B105-02
Research Triangle Park, NC 27711

From: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Sent: Friday, April 9, 2021 7:43 AM
To: Gavett, Stephen <Gavett.Stephen@epa.gov>
Cc: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Preliminary notice of animal welfare issue

Thank you for this report, Dr. Gavett. 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: Gavett, Stephen <Gavett.Stephen@epa.gov>
Sent: Thursday, April 8, 2021 8:16 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; Hoopes, Maria <Hoopes.Maria@epa.gov>; Jarrell, Leslie <Jarrell.Leslie@epa.gov>; Hedge, Joan <Hedge.Joan@epa.gov>
Subject: Preliminary notice of animal welfare issue

To the Division of Compliance Oversight:

As chair of our IACUC, I am writing to provide preliminary notice of an adverse event impacting animal welfare which was identified Monday April 5, 2021, at our institution (US EPA, Office of Research and Development, Research Triangle Park, NC; OLAW Assurance number D16-00569, A4051-01).

One tank held 4 cylinders of 19 day old zebrafish (100 total), and one of the cylinders fell over into the tank allowing them to escape into the tank water. After transferring all fish from the tank to new cylinders and a new tank, the care staff came back and found all 100 dead. Physical trauma is not suspected as all were equally affected. The water quality parameters in the new tank were a bit off but not terribly so. We believe that the tank in which the zebrafish were transferred may have been contaminated with a residual chemical, most likely chlorine. We have taken steps to correct the situation so that tank surfaces are thoroughly cleaned and rinsed before use and stored away from other chemicals. A final report from our Institutional Official (Dr. Cascio) with corrective actions will be delivered to OLAW as soon as all the facts are gathered.

Please let me know if you need further information.

Sincerely,

Stephen Gavett, Ph.D., D.A.B.T.

Research Biologist
Cardiopulmonary and Immunotoxicology Branch
CIB PHITD CPHEA ORD USEPA
Tel. (b) (6)
Mailing Address:
U.S. Environmenal Protection Agency
Attn: Stephen Gavett
Mail Code: B105-02
Research Triangle Park, NC 27711